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FORM PTO-1390 (Modified) (REV 11-2000) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY'S DOCKET NUMBER	U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR
PU3513USW	10/031355

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/EP99/05271	23 July 1999	23 July 1999

TITLE OF INVENTION

COMBINATION OF AN ANTI-EP-CAM ANTIBODY WITH A CHEMOTHERAPEUTIC AGENT

APPLICANT(S) FOR DO/EO/US

Vincent C. KNICK; Julie Beth STIMMEL; Linda M. THURMOND

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (24) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (Article 31).
5. ☒ A copy of the International Application as filed (35 U.S.C. 371 (c) (2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U.S C. 154(d)(4).
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371 (c)(3))
   a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired.
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3))
9. ☒ An oath or declaration of the inventor(s) (35 U.S C. 371 (c)(4)).
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371 (c)(5)).
11. ☒ A copy of the International Preliminary Examination Report (PCT/IPEA/409).
12. ☒ A copy of the International Search Report (PCT/ISA/210).

Items 13 to 20 below concern document(s) or information included:

13. ☒ An Information Disclosure Statement under 37 CFR 1.97 and 1.98
14. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
15. ☒ A **FIRST** preliminary amendment.
16. ☐ A **SECOND** or **SUBSEQUENT** preliminary amendment.
17. ☐ A substitute specification.
18. ☐ A change of power of attorney and/or address letter.
19. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821 - 1.825.
20. ☐ A second copy of the published international application under 35 U S.C. 154(d)(4).
21. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
22. ☒ Certificate of Mailing by Express Mail
23. ☒ Other items or information:

Cover page of international publication
PCT Request

PCTUS1/REV03

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U.S. APPLICATION NO (IF KNOWN, SEE 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
10/031355	PCT/EP99/05271	PU3513USW

24. The following fees are submitted:.	CALCULATIONS PTO USE ONLY	
BASIC NATIONAL FEE (37 CFR 1.492 (a) (1) - (5)) :		
☐ Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO **$1040.00**		
☒ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO **$890.00**		
☐ International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO **$740.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) **$710.00**		
☐ International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) **$100.00**		
ENTER APPROPRIATE BASIC FEE AMOUNT =	**$890.00**	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (e)).	**$0.00**	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	15 - 20 =	0	x $18.00	**$0.00**	
Independent claims	5 - 3 =	2	x $84.00	**$168.00**	
Multiple Dependent Claims **(check if applicable)**.			☐	**$0.00**	
TOTAL OF ABOVE CALCULATIONS =				**$1,058.00**	
☐ Applicant claims small entity status. See 37 CFR 1.27) The fees indicated above are reduced by 1/2.				**$0.00**	
SUBTOTAL =				**$1,058.00**	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492 (f)). +				**$0.00**	
TOTAL NATIONAL FEE =				**$1,058.00**	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31) **(check if applicable).** ☐				**$0.00**	
TOTAL FEES ENCLOSED =				**$1,058.00**	
				Amount to be: refunded	$
				charged	$

a. ☐ A check in the amount of _____ to cover the above fees is enclosed.

b. ☒ Please charge my Deposit Account No. **07-1392** in the amount of **$1,058.00** to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. **07-1392** A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. **Credit card information should not be included on this form.** Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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PATENT TRADEMARK OFFICE

V. C Bennett
SIGNATURE

Virginia C. Bennett
NAME

37,092
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(54) Title: COMBINATION OF AN ANTI-EP-CAM ANTIBODY WITH A CHEMOTHERAPEUTIC AGENT

(57) Abstract: A combination of an anti-Ep-CAM antibody with a chemotherapeutic agent that is capable of arresting Ep-CAM antigen expressing cells in S or G_2/M.

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Title: *COMBINATION OF AN ANTI-EP-CAM ANTIBODY WITH A CHEMOTHERAPEUTIC AGENT*

Commissioner for Patents
Washington, D.C. 20231

Attention: Box PCT/DO/EO/US

FIRST PRELIMINARY AMENDMENT

Sir:

The above identified application is being transmitted herewith for entry into the U.S. National Phase under Chapter II of the PCT. For the purposes of adding the priority information, please amend the application as follows:

In the Abstract:

Please substitute the attached Abstract, which has been placed on a separate piece of paper according to US practice.

In the Specification:

On the first line of the specification, after the Title, please add:

--This application is filed pursuant to 35 U.S.C. § 371 as a United States National Phase Application of International Application No. PCT/EP99/05271 filed July 23 1999. --

In the Claims:

Please amend the claims as follows:

Clean Copy of Pending Claims

4. A combination according to claim 1 wherein the chemotherapeutic agent is one or more agents selected from UFT, Capecitabine, CPT-ll, Oxaliplatin, 5FU continuous infusion, Paclitaxel, Docetaxel, Cyclophosphamide, Methotrexate, Doxorubicin, Navelbine (iv and oral), Epirubicin, Mitoxantrone, Raloxifen, Cisplatin, Mitomycin, Carboplatinum, Gemcitabine, Etoposide and Topotecan.

6. A combination according to claim 1 wherein the Ep-CAM expressing cells are cells of epithelial origin.

7. A combination according to claim 1 wherein the Ep-CAM antigen expressing cells are tumour cells and their metastases.

9. A combination according to claim 7, wherein the Ep-CAM expressing cells are prostate, lung, breast, gastric or colon originating cells or other tumours known to express the Ep-CAM antigen.

REMARKS

Currently claims 1-15 are pending. Claims 4, and 6-7 have been amended to place them in form appropriate to US practice and to reduce the filing fee by removing multiple dependency. Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned **"Version With Markings To Show Changes Made"**. Applicants have attached an abstract on a separate sheet of paper as required by US practice. Applicants have amended the specification for purposes of adding the priority information.

Respectfully submitted,

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Date: 18 Jan. 2002
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ABSTRACT

COMBINATION OF AN ANTI-EP-CAM ANTIBODY WITH A CHEMOTHERAPEUTIC AGENT

A combination of an anti-Ep-CAM antibody with a chemotherapeutic agent that is capable of arresting Ep-CAM antigen expressing cells in S or G_2/M.

Version With Markings To Show Changes Made

4. A combination according to claim 1 [any of the above claims] wherein the chemotherapeutic agent is one or more agents selected from UFT, Capecitabine, CPT-11, Oxaliplatin, 5FU continuous infusion, Paclitaxel, Docetaxel, Cyclophosphamide, Methotrexate, Doxorubicin, Navelbine (iv and oral), Epirubicin, Mitoxantrone, Raloxifen, Cisplatin, Mitomycin, Carboplatinum, Gemcitabine, Etoposide and Topotecan.

6. A combination according to claim 1 [any of the above claims] wherein the Ep-CAM expressing cells are cells of epithelial origin.

7. A combination according to claim 1 [any of the preceding claims] wherein the Ep-CAM antigen expressing cells are tumour cells and their metastases.

9. A combination according to claim 7, [claims 7 and 8] wherein the Ep-CAM expressing cells are prostate, lung, breast, gastric or colon originating cells or other tumours known to express the Ep-CAM antigen.

COMBINATION OF AN ANTI-EP-CAM ANTIBODY WITH A CHEMOTHERAPEUTIC AGENT

This present invention relates to the combination of antibodies that specifically bind to the EP-CAM antigen with chemotherapeutic agents that affect cell growth by blocking progression of the cell cycle in G_2/M and their use in therapy of cancers which express the antigen.

The conventional therapeutic approaches to cancer include surgery, radiotherapy and chemotherapy in various combinations; however, response rates have not improved significantly in the last 20 years. The major limitation of chemotherapy and radiotherapy is the non-selective targeting of both normal and tumour cells that results in toxic side effects. In the search for less toxic and more specific treatment alternatives, various types of immunotherapy have been investigated. Among these modalities, strategies based on monoclonal antibodies have been applied to a broad spectrum of malignancies (Riethmüller et al. Curr Opin Immun 1992, 4, 647-655 and Riethmüller et al. Curr Opin Immunol 1993, 5, 732-739). The utility of monoclonal antibodies is based upon their clonal antigen specificity, i.e. molecular recognition of specific epitopes which may comprise an antigen and to bind to these antigens with high affinity. Monoclonal antibodies can bind to antigens expressed uniquely or preferentially on the surface of malignant cells, and hence can be used to specifically target and destroy tumour cells. Antibodies may be constructed as delivery vehicles for drugs or DNA, or as conjugates with radionuclides. Binding of naked antibody to target cells may also activate innate antitumour immune functions such as antibody-dependent cell-mediated cytotoxicity (ADCC) and complement-mediated cytotoxicity (CMC), either of which may result in lysis or phagocytosis of the targeted cell. Both ADCC and CMC are antibody-dose-related immune functions and it is therefore desirable to get as much antibody bound to target cells as possible. One way of achieving this objective is to increase the level of expression of the relevant antigen which may effectively increase antibody functions such as, for example, ADCC of the target cells by virtue of getting more antibody bound to the cells (Fogler et al. Cancer Research 48 : 6303-6308 (1988)).

One antigen of importance in cancer therapy is the Ep-CAM antigen (human pan-carcinoma antigen). This antigen is a transmembrane glycoprotein which has been reported to function as a cell adhesion molecule (Litvinow et al. J. Cell Biology 125: 437-446, 1994) and is also known as the 17-1A antigen, 40kD antigen, EGP40, GA733-2, KSA and ESA but may be known by other names or descriptions in the literature as well. It is expressed on the baso-lateral surface of a majority of simple cuboidal or columnar, pseudo stratified columnar and transitional epithelia and at generally higher levels in tumour cells. Epithelial cells are known to be the most important cell type in the development of human malignancies. Thus more than 90% of all malignant tumours are carcinomas, and therefore of epithelial origin (Acta Anatomica; 156 (3); 217-226 (1996)). Although the Ep-CAM antigen is expressed on most tumour cells of epithelial origin there are examples of cells of epithelal origin that do not express Ep-CAM such as adult epithelial tissues, epidermal adult keratinocytes, gastric parietal cells, thymic cortical epithelium, myoepithelial cells and hepatocytes. The phenotype of a malignant cell plays an important role in the efficacy of monoclonal antibodies. While tumour specific antigens have proven to be elusive, differences in expression of the antigens between normal cells and tumour cells have provided a means to target antibodies to tumours. It would be clinically advantageous to improve on these differences by enhancement of antigen homogeneity and density of expression on tumour cells.

Interferons are well-known to alter cell phenotypes by increasing expression of tumour antigens as well as many normal antigens, e.g. Class I HLA. For example, human recombinant interferon-α and interferon-γ can increase the expression of human tumour antigens TAG-72 and CEA (Greiner et al. Cancer Res 44:3208-3214 (1984)). Interferon exposure induced a more homogeneous CEA-positive tumour cell population which shed more CEA from the cells surface, which was confirmed by *in vivo* studies with human carcinoma xenografts in athymic mice. Treatment with interferon-γ enhanced TAG-72 and CEA expression on ovarian or gastrointestinal tumour cells in patients' malignant ascites (Greiner et al. J Clin Oncol 10:735-746 (1992)). The effects of interferons on cells are myriad and range from direct cytotoxicity to immunopotentiation to antiproliferative activity. None of the effects of interferons

on antigen expression have been directly ascribed to interference with cell cycle progression.

Briefly, cell cycle progression refers to the sequence of events between one mitotic division and another in a cell. A quiescent resting phase (G_0) is followed by a growth phase (G_1), then by a DNA synthesis phase (S). A second growth phase of cell enlargement (G_2) and DNA replication (M phase) is followed by division of the cell into two progeny cells. Any interference with the cell machinery may inhibit all cycle progression at any phase of the cell cycle. For example, specific chemotherapeutic agents may block progression in either G_2 or M or in both G_2 and M (G_2/M). In other words exposure to certain drugs e.g. chemotherapeutic agents will for example, arrest individual cells in G_2 and/or M until eventually most, or all of the cells in a population become arrested in G_2 and/or M (G_2/M). In HeLa cells, for example, the G_1, S, G_2 and M phase take 8.2, 6.2, 4.6 and 0.6 hours, respectively. The period between mitoses is called interphase. Cells may have different doubling times, depending on their developmental stage or tissue type. The variation in doubling times is usually a function of the time spent in G_1 (A Dictionary of Genetics, 5th edition , RC King and WD Stansfield, Oxford University Press,1997).

While a few proteins have been identified as produced solely at certain phases of the cell cycle, and therefore can serve as markers of cell cycle status, most others are produced across the cell cycle but at higher or lower levels at certain points. Variation of antigen density across the cell cycle is typical for the sarcoma antigens p102 and p200 (Song S, Anticancer Research 16(3A) : 1171-5 (1996)), the leukaemia/lymphoma-associated antigen JD118 (Czuczman et al. Cancer Immunology, Immunotherapy 36(6):387-96 (1993)), and the gastric tumour antigen PC1 (Wei et al., J of Oncology 9(3) : 179-82 (1987)). A few tumour antigens have been reported to be cell-cycle independent, e.g. liver metastases 3H4 (Wulf et al., J. Cancer Research and Clinical Oncology 122(8) : 476-82 (1996)) and small cell lung cancer antigens (Fargion et al., Cancer Research 46 : 2633-2638 (1986)).

Surprisingly, it has been found that pre-treatment with a drug, for example a chemotherapeutic agent known to block cell cycle progression at S and/or G_2/M results in a significant increase in the density of the Ep-CAM antigen population

and thus in greater targeting of anti-Ep-CAM antibodies to Ep-CAM expressing tumours. In lytic antibodies this translates into an increased susceptibility to antibody-dependent cytolysis. This perturbation of tumour cell phenotype has a significant impact on the biological effectiveness of therapeutic antibodies, and provides synergistic benefit to standard chemotherapeutic regimens. Furthermore, this increase in Ep-CAM antigen expression appears to be tumour specific. In other words, pre-treatment with chemotherapeutic agents that block the cell cycle at S and/or G_2/M does not seem to affect Ep-CAM antigen expression in non-tumour cells.

Accordingly, the present invention provides a combination of an Ep-CAM antibody and a chemotherapeutic agent that is capable of arresting Ep-CAM antigen expressing cells in S or G_2/M, preferably in G_2/M.

Examples of anti-Ep-CAM antibodies are ING1 (Colcher et al., Proc. Natl. Acad. Sci. USA, 78 (5), 3199 to 3203 (1981) and Laio et al, Human Antibody Hybridomas 1(2), 66 -76 (1990)); 17-1A e.g. Panorex (Herlyn et al, Proc. Natl. Acad. Sci. USA 76 : 1438 - 1452 (1979) and Herlyn et al, Hybridoma 1985; 5 (suppl. 1) S3 to S10); and NR-LU-10 (Okabe et al, Cancer Research, 44, 5273 to 5278 (1984)).

Panorex (Adjuqual®) is a 17.1A mouse monoclonal antibody. It is marketed by Glaxo Wellcome in Germany for the post-operative adjuvant therapy of colorectal cancer.

An example of an anti-Ep-CAM antibody is one with the variable light chain cDNA sequence as set out in Figure 15 and the heavy chain cDNA sequence as set out in Figure 16. (known as humanised 323/A3/IgG_1). Two further preferred examples of anti- Ep-CAM antibodies are those with the variable light chain cDNA sequence as set out in Figures 15 and heavy chain cDNA sequences as set out in Figures 17 or 18 respectively (known as humanised 323/A3 IgG_4 and IgG_2cys respectively).

A preferred example of an anti-Ep-CAM antibody is 17.1A, most preferably Panorex.

Specific anti-Ep-CAM antibodies can be given on their own or in combination with other anti-Ep-CAM antibodies. Examples of such anti-Ep-CAM antibody combinations are an anti-Ep-CAM antibody with the variable light chain cDNA sequence as set out in Figure 15 and the heavy chain cDNA sequence as set out in Figure 16 in combination with ING1. Thus throughout the specification reference to an anti-Ep-CAM antibody includes antibody combinations of various anti-Ep-CAM antibodies, preferably non-competing anti-Ep-CAM antibodies targeting different epitopes on the same Ep-CAM antigen.

Examples of chemotherapeutic agents which are capable of arresting Ep-CAM antigen expressing cells in G_2/M are vinorelbine, cisplatin, mytomycin, paclitaxel, carboplatin, oxaliplatin and CPT-II (camptothecin).

Vinorelbine tartrate is a semisynthetic vinca alkaloid with the chemical name 3',4'-didehydro -4'-deoxy-C'-norvincaleukoblastine [R-(R*,R*)-2,3-dihydroxybutanedioate (1:2)(salt)]. Vinorelbine tartrate is used in combination with other chemotherapy agents such as cisplatin or as a single agent in the treatment of various solid tumours particularly non-small cell lung, advanced breast, and hormone refractory prostate cancers. The brand name Navelbine® is used in North America and Europe. Navelbine® is administered intravenously as a single-agent or in combination therapy typically at doses of 20-30 mg/m^2 on a weekly basis. An oral formulation of vinorelbine is in clinical development.

Cisplatin has the chemical name cis-diamminedichloroplatinum. Cisplatin is used in the treatment of metastatic testicular tumours as a combination therapy, as single and combination therapy in metastatic ovarian tumours, as well as a single agent in advanced bladder cancer. Cisplatin is manufactured by Bristol-Myers Squibb under the brand names of Platinol® and Platinol-AQ®. Cisplatin is also used in the following types of cancer, typically in combination therapy: non-small cell and small cell lung cancers, head and neck, endometrial, cervical, and non-Hodgkin's lymphoma. Cisplatin is typically administered intravenously in doses ranging from 15-150 mg/m^2 once every 3 to 4 weeks, or daily for 5 days repeated every 3 or 4 weeks. However, higher and more frequent doses are occasionally administered and the route of administration could be different than intravenous, such as intra-arterial or intraperitoneal.

Carboplatin has the chemical name platinum, diammine [1,1-cyclobutane-dicarboxylato(2)-0,0']-(SP-4-2). Carboplatin is usually administered in combination with other cytotoxics such as paclitaxel and etoposide. It is used in the treatment of advanced ovarian cancer, non-small cell lung cancer as well as in many of the same types of cancer as cisplatin is used. The brand name of carboplatin manufactured by Bristol-Myers Squibb is Paraplatin®. Carboplatin is typically administered intravenously at 300 - 400 mg/m^2, or to a target area under the drug concentration versus time curve (AUC) of 4-6 mg/ml-min using the patient's estimated glomerular filtration rate (GFR). Higher doses up to around 1600 mg/m^2 divided over several, usually five, days may also be administered.

Paclitaxel has the chemical name 5β, 20 epoxy-1,2α,4,7β,10β,13α-hexahydroxytax-11-en-9-one 4,10-diacetate 2-benzoate 13-ester with (2*R*, 3*S*)-*N*-benzoyl-3-phenylisoserine. Paclitaxel is manufactured by Bristol-Myers Squibb as Taxol®. It is used to treat a variety of carcinomas including ovarian, breast, non-small cell lung, head and neck. Typical doses include 135-175 mg/m^2 as either a 3 or 24 hour intravenous infusion given every 3 or 4 weeks. Higher doses up to around 300 mg/m^2 have also been administered.

Besides the active ingredient, the drug products provided by manufacturers typically contain a diluent such as sterile water, dextrose 5% in water or 0.9% sodium chloride in water with additional excipients such as Cremophor vehicle added to make for example, paclitaxel soluble.

More detailed information on treatment regimes, dosages and compositions etc can be obtained from standard reference books such as: Martindale, The Extra Pharmacopoeia, 31st edition, edited by JEF Reynolds, London, Royal Pharmaceutical Society, 1996 and the Physicians Desk reference, 49th Edition, 1995, Medical Economics Data Production Company, Montvale.

Other chemotherapeutic agents that may cause cells to accumulate in G_2 /M include anthracyclines e.g. doxorubicin and aclarubicin; carmustine (BCNU), camptothecin, 9-nitro-camptothecin, cyclophosphamide and its derivatives,

docetaxel, etoposide, Razoxane (ICRF-187), alkyllyso-phospholipids e.g. ilmofosine; methotrexate, MST-16, taxanes, vinblastine, vincristine and teniposide (VM-26) (again see Martindale, The Extra Pharmacopoeia, 31st edition, edited by JEF Reynolds, London, Royal Pharmaceutical Society, 1996,) and flavonoids e.g. apigenin and genistein (see The Merck Index, 12th edition, Merck Research Laboratories, Merck and Co Inc, 1996). In addition, adozelesin (a class of pyrazole compounds) (Cancer Research 1992, October 15; 52 (2) : 5687 to 5692)), Bistratene A (Mutation Research 1996, March 1; 367 (3) : 169 to 175), cycloxazoline (Cancer Chemotherapy & Pharmacology 1994; 33(5) : 399 to 409), imidazoarcridinone, melephan (Experimental Cell Biology 1986; 54 (3) : 138 to 148 and International Journal of Cancer 1995, Jul 17; 62 (2) : 170 to 175), merbarone (Environmental & Molecular Mutagenesis 1997; 29 (1) : 16 to 27 and Cancer Research 1995, Apr 1; 55 (7) : 1509 to 1516) and oracin (FEBS Letters 1997, Jan 2; 400 (1) : 127 to 130) are also believed to cause cells to accumulate in G_2/M generally all topo II inhibitors, e.g. to potecan (abpi, 1998-1999), all vinca derivatives and all DNA damaging agents including radiation are also believed to arrest cells in G_2/M.

Moreover, 5FU has been reported to arrest cells in G_2/M (Oncology Research 1994; 6(7):303-309) and it is therefore believed that 5FU and compounds similar to 5FU such as UFT, methotrexate, capecitabine and Gemcitabine will increase Ep-Cam expression in some tissues. Similarly, tomudex (Raloxifen) which is known to arrest cells in the S phase is believed to increase Ep-Cam expression.

The term "chemotherapeutic agent" throughout the specification is therefore not limited to cytotoxic therapy, but also encompasses cytostatic therapy and any other drugs capable of stopping cells in G_2/M. It should be further noted that radiotherapy is capable of arresting cells in G_2/M and that throughout the specification the term chemotherapeutic can therefore be substituted with "radiotherapy".

Throughout the specification reference to a chemotherapeutic agent includes combinations of one or more specific chemotherapeutic agents which arrest Ep-CAM expressing tumour cells in G_2/M. Examples of typical combinations are vinorelbine with cisplatin and paclitaxel with carboplatin; oxaliplatin with 5FU;

cyclophosphamide with methotrexate and 5FU; cyclophosphamide with doxorubicin and 5FU.

While it is possible for the chemotherapeutic agent to be administered alone it is preferable to present it as a pharmaceutical composition comprising an active ingredient, as defined above, together with an acceptable carrier therefor. Each carrier must be "acceptable" in the sense of being compatible with the other ingredients of the composition and not injurious to the recipient.

Preferred combinations of an Ep-Cam antibody and a chemotherapeutic agent(s) that are capable of arresting Ep-CAM antigen expressing cells in S or G_2/M are: Panorex in combination with any of the following chemotherapeutic agents: UFT, Capecitabine, CPT-II, Oxaliplatin, 5FU, 5FU continuous infusion, Paclitaxel, Docetaxel, Cyclophosphamide, Methotrexate, Doxorubicin, Navelbine (iv and oral), Epirubicin, Mitoxantrone, Raloxifen, Cisplatin, Mitomycin, Carboplatinum, Gemcitabine, Etoposide and Topotecan.

Particularly preferred combinations are Panorex with CPT-II, 5FU (continuous infusion), Oxaliplatin, Capecitibine, UFT and Tomudex (Raloxifen).

These Panorex combinations are useful in the treatment of cancer, particualrly in the treatment of colorectal cancer, breast cancer, gastric cancer, prostate cancer and non-small-cell lung cancer.

Specifically, the following combinations are particualrly preferred for colorectal cancer: Panorex in combination with: UFT (optionally with Leucovorin); Capecitabine; Oxaliplatin (optionally with 5FU); CPT-II, 5FU (optionally with Eniluracil or Levamisole or Leucovorin); 5FU protacted continuous infusion; and Mitomycin.

Preferred combinations for the treatment of breast cancer are: Panorex in combination with Paclitaxel; Docetaxel; Cyclophosphamide (optionally with 5FU and either Methotrexate or Doxorubicin); Navelbine (iv and/or oral); Doxorubicine; Epirubicin; Mitoxantrone; and Raloxifin.

Preferred combinations for the treatment of gastric cancer are: Panorex in combination with Cisplatin; 5FU; Mitomycin; and Carboplatinum.

A preferred combination for the treatment of prostatic cancer is: Panorex in combination with Mitoxantrone.

Preferred combinations for the treatment of non-small-cell lung cancer are: Panorex in combination with: Navelbine; Cisplatin; Carboplatin; Paclitaxel; Docetaxel; Gemcitabine; Topotecan; and Etoposide.

Information regarding dosing of Panorex and the above chemotherapeutic agents given in combination with Panorex can be found in standard reference books such as ABPI, Compendium of Data Sheets and Summaries of Product Characteristics, Datapharm Publications Limited, 1998-1999.

The compositions include those suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal, parenteral (including subcutaneous, intramuscular, intravenous and intradermal) or transdermal administration. The compositions may conveniently be presented in unit dosage form and may be prepared by any methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general, the compositions are prepared by uniformly and intimately bringing into association the active ingredient with liquid carriers or finely divided solid carriers or both, and then if necessary shaping the product.

Compositions of the chemotherapeutic agent suitable for oral administration may be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the active ingredient; as a powder or granules; as a solution or suspension in an aqueous or non-aqueous liquid; or as an oil-in-water liquid emulsion or a water-in-oil liquid emulsion. The active ingredient may also be presented as a bolus, electuary or paste.

A tablet may be made by compression or moulding, optionally with one or more accessory ingredients. Compressed tablets may be prepared by compressing in

a suitable machine the active ingredient in a free-flowing form such as a powder or granules, optionally mixed with a binder (e.g. povidone, gelatin, hydroxypropylmethyl cellulose), lubricants, inert diluent, preservative, disintegrant (eg. sodium starch glycollate, cross-linked povidone, cross-linked sodium carboxymethyl cellullose) surface-active or dispersing agent. Moulded tablets may be made by moulding in a suitable machine a mixture of the powdered compound moistened with an inert liquid diluent. The tablets may optionally be coated or scored and may be formulated so as to provide slow or controlled release of the active ingredient therein using, for example, hydroxypropylmethyl cellulose in varying proportions to provide the desired release profile. Tablets may optionally be provided with an enteric coating to provide release in parts of the gut other than the stomach.

Compositions suitable for oral use as described above may also include buffering agents designed to neutralise stomach acidity. Such buffers may be chosen from a variety of organic or inorganic agents such as weak acids or bases admixed with their conjugated salts.

Composition suitable for topical administration in the mouth include lozenges comprising the active ingredient in a flavoured basis, usually sucrose and acacia or tragacanth; pastilles comprising the active ingredient in an inert basis such as gelatine and glycerin, or sucrose and acacia and mouthwashes comprising the active ingredient in a suitable carrier.

Compositions for rectal administration may be presented as a suppository with suitable base comprising for example cocoa butter or a salicylate.

Compositions suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or spray formulations containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

Compositions suitable for parenteral administration include aqueous and non-aqueous isotonic sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the compositions isotonic with

the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents, such as liposomes or other microparticulate systems which are designed to target the compounds to blood components or one or more organs. The compositions may be presented in unit-dose or multi-dose sealed containers, for example, ampoules and vials, and may be stored in a freeze-dried(lyophilized) condition requiring only the addition of sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Compositions suitable for transdermal administration may be presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time. Such patches suitably contain the active ingredient as an optionally buffered, aqueous solution of, for example, 0.1 0.2M concentration with respect to said compound. As one particular possibility, the active ingredient may be delivered from the patch by iontophoresis as generally described in Pharmaceutical Research, 3 (6),318 (1986).

It should be understood that in addition to the ingredients particularly mentioned above the compositions in question, for example, those suitable for oral administration may include such further agents as sweeteners, thickeners and flavouring agents.

The dosage range of the chemotherapeutic agent to be co-administered with the antibody may typically be between about 1 to 1000 mg/m^2 (based on patient body surface area) or about 2 - 30 mg /kg (based on patient body weight), depending on the chemotherapeutic agent(s) used. Thus, for example, vinorelbine (navelbine) would typically be administered at a dosage of about 20 to 30 mg/m^2, cisplatin at about 15 to 100mg/m^2 carboplatin at about 300 to 600 mg/m^2 and paclitaxel at about 100 to 300 mg/m^2, preferably around 135 to 175 mg/m^2. Another way of expressing dosage is by their AUC value. For example carboplatin would typically be administered at a dose calculated as AUC = 4 to 6mg/ml-min. Generally, the doses of chemotherapeutic agents are lower when given in combination with another chemotherapeutic agent and/or antibody than if given on their own as the single chemotherapeutic agent. The doses of

chemotherapeutic agents that will be co-administered with anti Ep-CAM antibody(ies) will likely be the standard doses for the type of carcinoma treated or lower doses. In general the highest tolerated doses of the chemotherapeutic agents are administered either alone or in combination.

The anti-Ep-CAM antibodies of the present invention preferably have the structure of a natural antibody or a fragment thereof. Antibodies typically comprise two heavy chains linked together by disulphide bonds and two light chains. Each light chain is linked to a respective heavy chain by disulphide bonds. Each heavy chain has at one end a variable domain followed by a number of constant domains. Each light chain has a variable domain at one end and a constant domain at its other end. The light chain variable domain is aligned with the variable domain of the heavy chain. The light chain constant domain is aligned with the first constant domain of the heavy chain. The constant domains in the light and heavy chains are not involved directly in binding the antibody to antigen.

The variable domains of each pair of light and heavy chains form the antigen binding site. The domains on the light and heavy chains have the same general structure and each domain comprises a framework of four regions, whose sequences are relatively conserved, connected by three complementarity determining regions (CDRs). The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases forming part of the beta-sheet structure. The CDRs are held in close proximity by the framework regions and with the CDRs from the other domain, contribute to the formation of the antigen binding site, which in the case of the present invention is the formation of an anti-Ep-CAM binding site. CDRs and framework regions of antibodies may be determined by reference to Kabat et al ("Sequences of proteins of immunological interest" US Dept. of Health and Human Services, US Government Printing Office, 1987).

The preparation of an antibody in which the CDRs are derived from a different species than the framework of the antibody's variable domains is disclosed in EP-A-0239400. The CDR's may be derived from a rodent or primate monoclonal antibody. The framework of the variable domains and the constant

domains of such altered antibodies are usually derived from a human antibody. Such a humanised antibody should not elicit as great an immune response when administered to a human compared to the immune response mounted by a human against a wholly foreign antibody such as one derived from a rodent.

The antibody preferably has the structure of a natural antibody or a fragment thereof. Throughout the specification reference to antibody therefore comprises not only a complete antibody but also fragments such as a (Fab') 2 fragment, a Fab fragment, a light chain dimer or a heavy chain dimer. The antibody may be an IgG such as IgG_1, IgG_2, IgG_3 or IgG_4; or IgM, IgA, IgE or IgD or a modified variant thereof, including those that may be conjugated to other molecules such as radionuclides, enzymes etc. Typically, the constant region is selected according to the functionality required. Normally an IgG1 will demonstrate lytic ability through binding to complement and will mediate ADCC (antibody dependent cell cytotoxicity). An IgG_4 antibody will be preferred if a non-cytotoxic antibody is required. Antibodies according to the present invention also include bispecific antibodies such as, for example, the 17-1A antibody disclosed in Mack et al, The Journal of Immunology, 1997, 158 : 3965 -3970. Antibodies of the present invention may be murine, chimaeric or humanised with the preferred antibody being humanised antibody.

There are four general steps to humanise a monoclonal antibody. These are :

(1) determining the nucleotide and predicted amino acid sequence of the starting antibody light and heavy variable domains ;

(2) designing the humanised antibody, i.e. deciding which antibody framework region to use during the humanising process ;

(3) the actual humanising methodologies/techniques; and

(4) the transfection and expression of the humanised antibody.

More specifically,

Step 1 : Determining the nucleotide and predicted amino acid sequence of the antibody light and heavy chain variable domains

To humanise an antibody only the amino acid sequence of the antibody's heavy and light chain variable domains needs to be known. The sequence of the constant domains is irrelevant because these do not contribute to the reshaping strategy. The simplest method of determining an antibody variable domain amino acid sequence is from cloned cDNA encoding the heavy and light variable domain.

There are two general methods for cloning a given antibody's heavy and light chain variable domain cDNAs: (1) via a conventional cDNA library, or (2) via the polymerase chain reaction (PCR). Both of these methods are widely known. Given the nucleotide sequence of the cDNAs, it is a simple matter to translate this information into the predicted amino acid sequence of the antibody variable domains.

Step 2 : Designing the humanised antibody

There are several factors to consider in deciding which human antibody sequence to use during the humanisation. The humanisation of light and heavy chains are considered independently of one another, but the reasoning is basically similar for each.

This selection process is based on the following rationale : a given antibody's antigen specificity and affinity is primarily determined by the amino acid sequence of the variable region CDRs. Variable domain framework residues have little or no direct contribution. The primary function of the framework regions is to hold the CDRs in their proper spatial orientation to recognise the antigen. Thus the substitution of rodent CDRs into a human variable domain framework is most likely to result in retention of their correct spatial orientation if the human variable domain framework is highly homologous to the rodent variable domain from which they originated. A human variable domain should preferably be chosen therefore that is highly homologous to the rodent variable domain(s).

A suitable human antibody variable domain sequence can be selected as follows :

1. Using a computer program, search all available protein (and DNA) databases for those human antibody variable domain sequences that are most homologous to the rodent antibody variable domains. The output of a suitable program is a list of sequences most homologous to the rodent antibody, the percent homology to each sequence, and an alignment of each sequence to the rodent sequence. This is done independently for both the heavy and light chain variable domain sequences. The above analyses are more easily accomplished if only human immunoglobulin sequences are included.

2. List the human antibody variable domain sequences and compare for homology. Primarily the comparison is performed on lengths of CDRs, except CDR 3 of the heavy chain which is quite variable. Human heavy chains and Kappa and Lambda light chains are divided into subgroups; Heavy chain 3 subgroups, Kappa chain 4 subgroups, Lambda chain 6 subgroups. The CDR sizes within each subgroup are similar but vary between subgroups. It is usually possible to match a rodent antibody CDR to one of the human subgroups as a first approximation of homology. Antibodies bearing CDRs of similar length are then compared for amino acid sequence homology, especially within the CDRs, but also in the surrounding framework regions. The human variable domain which is most homologous is chosen as the framework for humanisation.

Step 3 : The actual humanising methodologies/techniques

An antibody may be humanised by grafting the desired CDRs onto a human framework according to EP-A- 0239400.(see also P.T. Jones et al, Nature 321:522 (1986); L. Reichman et al, Nature 332 :323(1988); Verhoeyen M. et al, Science 239:1534 (1988) and J. Ellis et al, The Journal of Immunology, 155 :925-937(1995)). A DNA sequence encoding the desired reshaped antibody can therefore be made beginning with the human DNA whose CDRs it is wished

to reshape. The rodent variable domain amino acid sequence containing the desired CDRs is compared to that of the chosen human antibody variable domain sequence. The residues in the human variable domain are marked that need to be changed to the corresponding residue in the rodent to make the human variable region incorporate the rodent CDRs. There may also be residues that need substituting in, adding to or deleting from the human sequence.

Oligonucleotides are synthesised that can be used to mutagenise the human variable domain framework to contain the desired residues. Those oligonucleotides can be of any convenient size. One is normally only limited in length by the capabilities of the particular synthesiser one has available. The method of oligonucleotide-directed in vitro mutagenesis is well known.

Alternatively humanisation may be achieved using the recombinant polymerase chain reaction (PCR) methodology of WO92/07075. Using this methodology, a CDR may be spliced between the framework regions of a human antibody.

In general, the technique of WO92/07075 can be performed using a template comprising two human framework regions, AB and CD and between them, the CDR which is to be replaced by a donor CDR. Primers A and B are used to amplify the framework region AB, and primers C and D used to amplify the framework region CD. However, the primers B and C each also contain, at their 5' ends, an additional sequence corresponding to all or at least part of the donor CDR sequence. Primers B and C overlap by a length sufficient to permit annealing of their 5' ends to each other under conditions which allow a PCR to be performed. Thus, the amplified regions AB and CD may undergo gene splicing by overlap extension to produce the humanised product in a single reaction.

Step 4 : The transfection and expression of the reshaped antibody

Following the mutagenesis reactions to reshape the antibody, the mutagenised DNAs can be linked to an appropriate DNA encoding a light or heavy chain constant region, cloned into an expression vector, and transfected into host

cells, preferably mammalian cells. These steps can be carried out in routine fashion. A reshaped antibody may therefore be prepared by a process comprising :

(a) preparing a first replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least a variable domain of an Ig heavy or light chain, the variable domain comprising framework regions from a human antibody and the CDRs required for the humanised antibody of the invention.

(b) preparing a second replicable expression vector including a suitable promoter operably linked to a DNA sequence which encodes at least the variable domain of a complementary Ig light or heavy chain respectively;

(c) transforming a cell line with the first or both prepared vectors; and

d) culturing said transformed cell line to produce said altered antibody.

Preferably the DNA sequence in step (a) encodes both the variable domain and the or each constant domain of the human antibody chain. The humanised antibody can be recovered and purified. The cell line which is transformed to produce the altered antibody may be Chinese Hamster Ovary (CHO) cell line or an immortalised mammalian cell line, which is advantageously of lymphoid origin, such as a myeloma, hybridoma, trioma or quadroma cell line. The cell line may also comprise a normal lymphoid cell, such as a B-cell, which has been immortalised by transformation with a virus, such as the Epstein-Barr virus. Most preferably, the immortalised cell line is a myeloma cell line or a derivative thereof. The expression system of choice is the glutamine synthetase expression system described in WO87/00462 (see also P.E. Stephens et al, Nucleic Acid Res. 17:7110 (1989) and C.R. Bebbington et al, Bio/Technology 10:169 (1992)).

Although the cell line used to produce the humanised antibody is preferably a mammalian cell line, any other suitable cell line, such as a bacterial cell line or a

yeast cell line, may alternatively be used. For single antibody chains, it is envisaged that E. coli - derived bacterial strains could be used. The antibody obtained is checked for functionality. If functionality is lost, it is necessary to return to step (2) and alter the framework of the antibody.

Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like (see generally Scopes, R, Protein Purification, Springer-Verlag, N.Y. (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, an antibody may then be used therapeutically.

Antibodies are typically provided as a pharmaceutical composition comprising a pharmaceutically acceptable carrier or diluent and, as active ingredient, an antibody according to the invention. The antibody and pharmaceutical compositions thereof are particularly useful for parenteral administration i.e. subcutaneously, intramuscularly or intravenously.

The compositions for parenteral administration will commonly comprise a solution of the antibody or a cocktail thereof dissolved in an acceptable carrier, preferably an aqueous carrier. A variety of aqueous carriers can be used, eg. sterile water for injection, 0.9% sodium chloride in water, 5% dextrose in water and Lactated Ringers solution. These solutions are sterile and generally free of particulate matter. These compositions may be sterilised by conventional, well known sterilisation techniques. The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, toxicity adjustment agents and the like, for example sodium acetate, sodium chloride, potassium chloride, calcium chloride, sodium lactate, etc. The concentration of antibody in these formulations can vary widely, for example from less than about 0.5%, usually at or at least about 1% to as much as 15 or 20% by weight and will be selected

primarily based on fluid volumes, viscosities, etc. in accordance with particular mode of administration selected.

Thus, a typical pharmaceutical composition for intramuscular injection could be made to contain 1 ml sterile buffered water, and 50 mg of antibody. A typical composition for intravenous infusion could be made up to contain 250 ml of sterile Ringers solution and 150mg of antibody. Actual methods for preparing parenterally administrable compositions will be known or apparent to those skilled in the art, particularly, those trained in the preparation of parenteral products and are described in more detail in, for example, Remmington's Pharmaceutical Science, 15th Ed., Mack Publishing Company, Easton, Pennsylvania (1990).

The antibodies of this invention can be lyophilised for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional immunoglobulins. Any suitable lyophilisation and reconstitution techniques can be employed. It will be appreciated by those skilled in the art that lyophilization and reconstitution can lead to varying degrees of antibody activity loss (eg. with conventional immune globulins, IgM antibodies tend to have greater activity loss than IgG antibodies) and that use levels may have to be adjusted to compensate.

The dosage range of the antibody in accordance with the invention is about 0.5 to 1000 mg/m^2, preferably about 0.5 to 250mg/m^2, more preferably, between 0.5 and 100mg/m^2 and 0.5 and 50mg/m^2 and most preferably between 5 and 25mg/m^2 such as for example, 15mg/m^2.

Similarly, expressed in mg per dose, the dosages of the antibody may be about 1 to 2000 mg per dose, preferably about 1 to 500 mg per dose, more preferably between 1 to 200 mg per dose and between 1 to 100mg per dose and most preferably between 10 and 50mg per dose such as, for example 30 mg per dose.

Single or multiple administrations of the compositions can be carried out with dose levels and pattern being selected by the treating physician. In any event,

the pharmaceutical formulations should provide a quantity of the antibody(ies) sufficient to effectively treat the patient.

Typically, the chemotherapeutic agent and antibody will be presented as separate pharmaceutical compositions for co- administration, but they may also be formulated as a single pharmaceutical formulation. In this way both the antibody and the chemotherapeutic agent are presented to the patient within one and the same composition.

One or more distinct chemotherapeutic agents and one or more distinct anti-Ep-CAM antibodies may be co-administered in all aspects of the present invention. Thus reference to a chemotherapeutic agent comprises one or more distinct chemotherapeutic agent(s). If there is more than one chemotherapeutic agent, these may be administered either individually each on its own and/or together as a chemotherapeutic agent cocktail. Similarly, reference to antibody comprises one or more distinct anti-Ep-CAM antibody(ies). If there is more than one antibody, these may again be administered either individually each on its own and/ or together as a cocktail. Additionally, the chemotherapeutic agent(s) are typically administered separately from the antibody(ies) but they may also be administered together as a chemotherapeutic agent(s)/antibody(ies) cocktail.

Co-administration of the chemotherapeutic agent/radiotherapy with the antibody comprehends administration substantially simultaneously of both the chemotherapeutic agent/radiotherapy and the antibody. Essentially, the rational behind co-administration is to allow sufficient exposure of Ep-CAM expressing tumour cells to a chemotherapeutic agent/radiotherapy known to block cell cycle progression at G_2 /M to achieve the desired increase in Ep-CAM antigen density prior to exposure of the same tumour cells to an anti-Ep-CAM antibody thereby achieving greater targeting of anti-Ep-CAM antibodies to Ep-CAM expressing tumours. Co-administration therefore comprises any mode of administering a chemotherapeutic agent/radiotherapy in conjunction with an anti-Ep-CAM antibody that will achieve this result. Throughout the specification the term "combination of an anti-Ep-CAM antibody with a chemotherapeutic agent" refers to one wherein the chemotherapeutic agent/radiotherapy and the anti-Ep-CAM antibody have been co-administered.

Preferably the chemotherapeutic agent is administered simultaneously with the antibody or more preferably before the antibody. Thus the chemotherapeutic agent may be administered on the same day as the antibody, either together or within hours of each other but may also be administered up to about two months beforehand, typically, about one or two weeks beforehand and more typically less than a week beforehand, say one to three days beforehand.

Additionally, co-administration also includes administering more than one dose of antibody within several weeks after one or more doses of chemotherapeutic agent, in other words the chemotherapeutic agent need not be re-administered again with every subsequent administration of the antibody, but may be administered just once or intermittently during the course of antibody treatment. Co-administration also comprises administration of the chemotherapeutic agent up to 3 weeks after the antibody, preferably within a week and more preferably within a few days such as one to five days.

The antibody may be administered several times daily. Similarly the chemotherapeutic agent may be infused continuously over several hours or even days.

The present invention also provides a method of treating mammalian patients, preferably humans, afflicted with cancer which comprises co-administering a chemotherapeutic agent which is capable of arresting Ep-CAM antigen expressing cells in G_2/M in combination with an anti-Ep-CAM antibody. Preferably, the chemotherapeutic agent is given simultaneously and more preferably prior to administration of the antibody.

The cancers which may be treated particularly effectively with this combination therapy are primary or metastatic cancers of any histologic or histogenetic origin that express the Ep-CAM antigen. This includes, for example, prostate cancers, lung cancers, breast cancers, colon cancers, pancreatic cancers and ovarian cancers.

Dosing schedules for the treatment method of the present invention can be adjusted to account for the patient characteristics, disease state, characteristics of the chemotherapeutic agent and characteristics of the anti-Ep-CAM antibody. The goal of dosing schedules under this invention will be to administer anti-Ep-CAM antibody in a manner that will expose the Ep-CAM expressing tumour cells to the anti-Ep-CAM antibody at a time when antigen expression is likely to be increased due to exposure to chemotherapy which is known to block cell cycle progression at G_2/M. Additionally, as much as possible a dosing schedule convenient for the patient must be maintained.

Preferred dosing schedules for administration of the anti-Ep-CAM antibody and chemotherapy include: administering the anti-Ep-CAM antibody once every one or two weeks, preferably once every three or four weeks or a combination thereof for as long as necessary. The chemotherapeutic agent is given according to the established regimen for that agent or a regimen which will allow exposure of Ep-CAM expressing tumour cells to be arrested in G_2/M. Preferred dosing schedules vary with the chemotherapy agent and disease state but include, for example, once weekly, once every three or four weeks, or daily for several (e.g. 3-5) days repeated every three or four weeks for as long as necessary. Dosing of the anti-Ep-CAM antibody may take place on the same day or different days as indicated for the chemotherapeutic agent. Adjustment of the dosing schedule or strength of dose to prevent or decrease toxicity or side effects may take place with either the anti-Ep-CAM antibody or the chemotherapy agent.

For example, the preferred dosing schedule for co-administration of vinorelbine and cisplatin in combination with humanised 323/A3 (IgG_1) is administration of humanised 323/A3 (IgG_1) at a dose of 30mg/m^2 once a week for as long as necessary but typically for a period of 3 to 4 weeks, followed by a 30mg/m^2 dose every other week thereafter for as long as necessary. Vinorelbine is administered at a dose 25mg/m^2 on day 1,8,15 and 22. Cisplatin is given only once at a dose of 100mg/m^2 on day 1. Thereafter the vinorelbine /cisplatin regime is repeated every 28 days for as long as necessary. Preferably, vinorelbine, cisplatin and humanised 323/A3 (IgG_1) are administered at the same time on day one over a period of about 2 to 3 hours.

Another example of a preferred dosing schedule is the administration of paclitaxel/carboplatin in combination with humanised 323/A3 (IgG_1), wherein 323/A3 (IgG_1) is administered as for the vinorelbine/cisplatin example above and paclitaxel and carboplatin are given at a dose of 225 mg/m^2 and AUC = 6.0 respectively, on day 1, with a repeat dosage every 28 days thereafter for as long as necessary. Again, paclitaxel, carboplatin and humanised 323/A3 (IgG_1) are preferably administered together on day 1 over a period of about 2 to 3 hours.

Other preferred dosage schedules which comprise the combination of 323/A3 (IgG_1) with any of navelbine, cisplatin or taxol on their own would comprise similar dosages and administration schedules, using just one anticancer agent instead of two.

When the preferred anti-Ep-CAM antibody is Panorex, the dosage of antiobdy is between 10 to 500mg per dose, preferably 100mg per dose.

A further aspect of the present invention is a method of increasing antibody binding of anti-Ep-CAM antibodies to Ep-CAM expressing cells by co-administering to a patient a chemotherapeutic agent capable of arresting cells in G_2/M together with said anti- Ep-CAM antibody.
By co-administering a chemotherapeutic agent according to the present invention together with an Ep-CAM antibody, it is possible to increase antibody binding by about 2 to 10 fold, preferably by more than 4 fold, more preferably by more than 6 fold and most preferably by more than 8 fold.

Figures

Figure 1.
Ep-CAM is expressed across the cell cycle, but at higher density and greater homogeneity on cells in S (dotted line) and in G_2/M (dashed line) phases than in G_0/G_1 cells (solid line). This pattern of expression has been documented in a number of other human colon, prostrate, and lung tumour cell lines.

Figure 2.
Cell cycle arrest is a prominent feature of adenocarcinoma cells exposed in vitro to Navelbine (NVB; 30 nM) plus Cisplatin (CDDP; 5 μM), or Taxol (TAX; 80 nM) plus Carboplatin (CPBDA; 100 μM), compared to media alone, 5-Fluorouracil (5FU), interferon-alpha (IFN-alpha; 100 U/ml), or interferon-gamma (IFN-gamma; 100 U/ml). The area of each bar is divided to indicate the percentage of cells in G_0/G_1 and in S + G_2/M phases; the height of each bar indicates the average number of Ep-CAM molecules per cell within the population. Cells in S phase and in G_2/M phase express higher levels of Ep-CAM (Figure 1), and the agents which blocked cell cycle progression had overall increased Ep-CAM expression

Figure 3.
The expression of Ep-CAM antigen was quantified on a variety of adenocarcinoma cell lines as well as primary cultures of normal human cells. Cultured cells were exposed sequentially to media, or to 30 nM Navelbine followed by 5 μM Cisplatin (NVB + CDDP), or to 80 nM Taxol followed by 100 μM Carboplatin (TAX + CPBDA). The 4 adenocarcinoma cells expressed higher antigen levels subsequent to exposure to cycle-specific drug combinations, whereas the 4 normal cells did not show any increase in antigen expression, which remained undetectable in 2 of the normal cell populations.

Figure 3a.
The binding of Panorex, a related murine monoclonal antibody with specificity for the Ep-CAM antigen, was evaluated after a 15 minute incubation with HT29 adenocarcinoma cells which had been cultured with Navelbine plus Cisplatin or with Taxol as previously described. A significant increase (34%) in antibody binding was seen on the cells treated with Navelbine plus Cisplatin; 82% of these cells were arrested in S or G_2/M cycle phase compared to 21% of the control cells. (A smaller increase (8%) in antibody binding was seen for cells treated with Taxol, but in this expeiment only 57% of the cells were cycle-arrested) as is shown in Figure 3a.

Figure 4.
The ability of human peripheral blood ADCC effector cells to lyse tumour target cells incubated with humanized 323/A3 (IgG_1) (a humanized monoclonal antibody having specificity for the Ep-CAM antigen and capable of interacting with Fc receptors on human effector cells) in vitro was improved when the target cells had been pre-treated with NAVELBINE (30 nM) plus Cisplatin (5 μM).

Figure 5.
Treatment of human tumour xenograft-bearing mice with a cell-cycle-specific cytotoxic agent promoted improved localization of antibody specific for Ep-CAM to the tumours.

Figure 6.
Humanised 323/A3 (IgG_1) Kappa Light Chain Amino Acid Sequence

Figure 7.
Humanised 323/A3 (IgG_1) Heavy Chain Amino Acid Sequence

Figure 8.
Vector Map of pEE6

Figure 9.
Vector Map of pEE12

Figure 10.
Vector Map of pEE18

Figure 11
Humanised 323/A3 (IgG_{4cys}) Kappa Light Chain Amino Acid Sequence

Figure 12
Humanised 323/A3 (IgG_{4cys}) variant Heavy Chain Amino Acid Sequence

Figure 13
Humanised 323/A3 (IgG_{2cys}) Kappa Light Chain Amino Acid Sequence

Figure 14
Humanised 323/A3 ($IgG_{2\,cys}$) Heavy Chain Amino Acid Sequence

Figure 15
Humanised 323/A3 (IgG_1) light chain cDNA Sequence

Figure 16
Humanised 323/A3 (IgG_1) Heavy chain cDNA Sequence

Figure 17
Humanised 323/A3 (IgG_4) heavy chain cDNA Sequence

Figure 18
Humanised 323/A3 (IgG_{2cys}) heavy chain cDNA Sequence

The following examples illustrate the invention.

Example 1. Ep-CAM antigen expression varied by phase across the cell cycle on PC-3 prostatic adenocarcinoma cells.

Populations of PC-3 prostatic adenocarcinoma cells were evaluated for distribution in G_0/G_1, S, and G_2/M phases of the cell cycle as well as Ep-CAM expression. Cells were gently trypsinized and mechanically detached from the culture flasks and resuspended in calcium-and magnesium-free phosphate-buffered saline containing bovine serum albumin and NaN_3. Exactly 2×10^5 cells were stained with FITC-323/A3 murine IgG antibody or FITC-murine IgG (control). Cells were fixed with cold paraformaldehyde, then permeabilized for DNA staining with Tween-20. Cellular DNA was stained with propidium iodide and RNase A. Listmode data were acquired on a FACScan flow cytometer (Becton Dickinson Immunocytometry Systems) equipped with a 488 nm laser using Cell Fit software. Cell cycle analysis was done using SOBR modelling (where possible, otherwise manual estimations were employed) on Cell Fit.

Ep-CAM antigen expression as detected by 323/A3 binding was evaluated separately using histogram analysis in Win List (Verity Software House).

Figure 1 shows that Ep-CAM is expressed across the cell cycle, but at higher density and greater homogeneity on cells in S (dotted line) and in G_2/M (dashed line) phases than in G_0/G_1 cells (solid line). This pattern of expression has been documented in a number of other human colon, prostate, and lung tumor cell lines.

Example 2. Increased expression of Ep-CAM antigen on adenocarcinoma cells was associated with arrest of cell cycle progression and accumulation of cells in S and G_2/M phases.

Adenocarcinoma cell lines were exposed to the various drugs or combinations of drugs as indicated in Figure 2. Subconfluent cells were exposed to Navelbine or Taxol for up to 24 hours, then washed and exposed to Cisplatin or Carboplatin, respectively, overnight. Cells were exposed to 5FU for 24 hours, and for 2-5 days to the interferons. Cells were washed and cultured for another 2-5 days prior to analysis for antigen expression and cell cycle status as described in Example 1. Antigen expression was quantified by comparison of the binding of fluorescein-conjugated 323/A3 to cultured cells with binding to calibrated microbead standards.

Cell cycle analysis demonstrated that only 6.3% of the media control cells were in S and G_2/M phases combined, compared to 39.4% of NVB + CDDP and 82.6% of TAX + CPBDA cells, both combinations of which caused significant increases in Ep-CAM antigen expression (as demonstrated in Figure 2). Antigen expression was not significantly increased in cells exposed to 5FU, IFN-α, or IFN-γ, which had only 7.9%, 12%, and 11.5%, respectively, of cells in S + G_2/M phase. Thus, only the drugs which caused accumulation of cells in S or G_2/M phases were able to cause a significant increase in Ep-CAM antigen expression.

Example 2a.

The binding of Panorex, a related murine monoclonal antibody with specificity for the Ep-CAM antigen, was evaluated after a 15 minute incubation with HT29 adenocarcinoma cells which had been cultured with Navelbine plus Cisplatin or with Taxol as previously described. A significant increase (34%) in antibody binding was seen on the cells treated with Navelbine plus Cisplatin; 82% of these cells were arrested in S or G_2/M cycle phase compared to 21% of the control cells. (A smaller increase (8%) in antibody binding was seen for cells treated with Taxol, but in this expeiment only 57% of the cells were cycle-arrested) as is shown in Figure 3a.

Example 3. Increased Ep-CAM antigen expression was observed on tumour cells but not normal cells exposed to cytotoxic drugs in vitro.

The expression of Ep-CAM antigen was quantified on a variety of adenocarcinoma cell lines as well as primary cultures of normal human cells. Cultured subconfluent cells were exposed sequentially to media, or to 30 nM Navelbine followed by 5 μM Cisplatin (NVB + CDDP), or to 80 nM Taxol followed by 100 μM Carboplatin (TAX + CPBDA). Cells were washed with media and cultured for another 2-5 days prior to analysis for antigen expression as described in Examples 1 and 2.

Figure 3 clearly shows that the 4 adenocarcinoma cells expressed higher antigen levels subsequent to exposure to cycle-specific drug combinations, whereas the 4 normal cells did not show any increase in antigen expression, which remained undetectable in 2 of the normal cell populations

Example 4. Cells exposed to NAVELBINE plus Cisplatin were better targets for human ADCC activity than control cells.

Adenocarcinoma cells were exposed to drugs as described in Examples 1 and 2 above, and then harvested and seeded into 96-well plates for use as target cells in a ^{51}Cr-release cytotoxicity assay. Target cells were cultured overnight

with ^{51}Cr, and then washed. Human peripheral blood mononuclear cells which had been allowed to adhere overnight were added at a 50:1 effector: target ratio, and the ADCC cultures were incubated for 6 hours. Supernatants were collected and counted for radioactivity, and the percentage specific release was calculated. (see Figure 4).

Figure 4 clearly shows that PC-3 prostatic adenocarcinoma cells are better targets for human ADCC activity after exposure to Navelbine/Cisplatin compared to controls which have not been exposed to these chemotherapeutic agents. This effect may be due directly to increased antigen expression and thereby increased antibody binding, decreased modulation of the Ep-CAM antigen, increased fragility of the target cells, or a combination of the above.

Example 5. Antibody targeting to Ep-CAM-positive tumours was significantly improved by pre-treatment of the mice with NAVELBINE.

Human colon adenocarcinoma (HT-29) tumours were initiated by subcutaneous implantation into female CD-1 nude mice (Charles River). When the tumours reached 200-300 mg, animals were divided into groups of five. Navelbine was injected intravenously at a dosage of 28 mg/kg on days 1 and 5. A control group was dosed with 5-fluorouracil (5-FU) intraperitoneally at 20 mg/kg on days 1 and 5. On day 6, humanised 323/A3 $IgG_{4cys\text{-}TMT}$ (a humanized monoclonal antibody chelator conjugate with specificity for the Ep-CAM antigen) was labelled with lutetium-177 and injected intravenously via the lateral tail vein. Each mouse received 4.1 μg protein/2.09 μCi lutetium-177/0.2 ml injection. Blood, spleen, liver, lung, kidney, femur and tumour were harvested on days 1, 3 and 5 post-antibody for direct gamma counting (see Figure 5 for results).

Figure 5 shows that pre-treatment with Navelbine increases antibody targeting to Ep-CAM positive tumours whilst pre-treatment with 5-FU does not.

Example 6. Expression of the Humanized Antibody 323/A3 (IgG_1) variant in NSO Cells

1. Purpose/Summary

The cDNAs encoding the humanized 323/A3 antibody light and heavy chains (see Figures 15 and 16 respectively) were genetically engineered into a single Celltech glutamine synthetase (GS) expression plasmid, pEE18 (see Fig. 10), and used to transfect murine NSO cells.

2. Materials and Methods

2.1 Materials

NSO cells were obtained from Celltech Biologics plc, Slough, SL1 4EN, Berkshire, UK. The expression plasmids pEE6HCMV and pEE12 (see Figures 8 and 9) were obtained from Celltech Biologics plc, Slough.

2.2 The pEE6hmcv plasmid (see Figure 8) encoding full length humanised heavy chain DNA was digested with *Bam HI* and *Bgl* II to liberate the 3.2 kb fragment that contained the DNA encoding the heavy chain under the transcriptional control of the major immediate early promoter of the human cytomegalovirus. This fragment was cloned into the *Bam HI* site of pEE12 (Figure 9) that contained the DNA encoding the humanised light chain. (See Figure 6 for humanised 323/A3 (IgG_1) Kappa light chain amino acid sequence and Figure 7 for the humanised 323/A3(IgG_1) Heavy chain amino acid sequence. See Figure 10 for schematic representation of the pEE18 plasmid encoding 323/A3 (IgG_1) heavy and light chains.

2.2.2 Transfection and Selection of NSO Cells

2.2.2.1 Tissue Culture

All single cell culture activities were performed in isolated rooms that contained a single laminar flow hood and single incubator dedicated solely to the use of NSO cells in the production of stable cell lines secreting humanised 323/A3(IgG_1). No other NSO cells lines, human

cell lines or virus transformed cell lines were used within this environment.

A vial of NSO cells was revived and grown in 1:1:1 medium composed of DMEM:RPMI-1640:Sigma PFHM (1:1:1) to a cell density between 0.5 and $1x10^6$mL. For electroporation, the cells were harvested by centrifugation and washed once with PBS. pEE18 plasmid DNA encoding 323/A3 (IgG_1) was digested with Sal I, heat inactivated at 65°C for 15 minutes, precipitated with ethanol and air-dried. The dried DNA pellet was resuspended in PBS to a concentration of 0.5 μg/mL and 100 μL aliquoted into a 2mm electroporation cuvette (BTX). Washed NSO cells were resuspended at 1.2 x 10^7/ml and 400 μL added to the cuvette to give a final density of 10^6 mL in a final volume of 0.5 mL. Electroporation was at 300 V for 1 msec in a BTX 8209 GenePulser followed by incubation on ice for 5-10 minutes. The electroporation mixture was resuspended at 10^5 cells /mL with 1:1:1 medium and distributed over 96-well plates at 50 μL/well. The following day, wells were fed with 150 μL GS medium (Gln-free IMDM, 1= X GS and nucleoside supplement, 5% DFBS) to begin the GS selection process such that all wells had a final concentration of 3% DFBS.

2.2.2.2 Specific Production Rate (SPR)

Selected cell lines grown in GS media (3% DFBS) were seeded at a density of 0.2 x 10^6 cells/mL in T-25 flasks (Costar) that contained 5 mL of GS media (3% DFBS). Cells were incubated overnight at 37°C for 24 hours after which an aliquot of each culture supernatant was removed. The supernatants were used in the human IgG ELISA assay to determine the concentration of secreted humanised 323/A3(IgG_1.). The SPR value was derived by multiplying the concentration of 323/A3 (IgG_1) antibody in the supernatant times the volume (5.0) and is expressed as μg/10^6 cells/24 hours.

2.2.2.3 Cryopreservation of Cells

Selected cell lines were routinely harvested when cell density was greater than 0.2 x 10^6 cells/mL. An appropriate volume of cells was removed and subjected to centrifugation at 1,000 x g for 5 minutes at 22°C. The cell pellet was gently resuspended to 1 - 4 x 10^6 cells/mL with ice-cold freezing media consisting of 20% (v/v) FBS/ 10-% (v/v) DMSO/ GS Media (sterile filtered). Each 1.0mL of the cell suspension was aliquoted into a 1.8 ml cryopreservation vial (NUNC) and gradually frozen overnight in a Cryo 1°C Freezing Container (Nalgene) that had been placed in a -70°C freezer. The vials were then removed from the container and stored in the vapour phase of a liquid nitrogen freezer.

Twenty vials of each cell line, including a low humanised 323/A3(IgG_1) producer were frozen down as described above and stored initially in the vapour phase of an MVE Cryogenics XLC440 liquid nitrogen freezer. The cells were subsequently transferred and stored in the vapour phase of an MVE Cryogenics XLC500 liquid nitrogen freezer.

Example 7. Expression of the Humanized Antibody 323/A3(IgG_{4cys}) in NSO Cells

1. Purpose Summary

The cDNAs encoding the humanized antibody 323/A3(IgG_{4cys}) (a humanised 323/A3 antibody) antibody light and heavy chains (see Figures 15 and 17 were genetically engineered into a single Celltech glutamine synthetase (GS) expression plasmid, pEE18, and used to transfect murine NSO cells.

2. Materials and Methods

2.1 Materials (as for Example 6 above)

2.2 Creation of humanised 323/A3 ($IgG_{4\,cys}$ pEE18 Expression Plasmid

The pEE6HMCV plasmid (see Figure 8) encoding full length humanized heavy chain DNA was digested with *BAM HI* and *Bgl II* to liberate a 3.2 kb fragment that contained the DNA encoding the heavy chain under the transcriptional control of the major immediate early

promoter of the human cytomeglovirus. This fragment was cloned into the *Bam HI* site of pEE12 that contained the DNA encoding the humanized light chain (See Figure 11 for humanised 323/A3(IgG_4) Kappa Light Chain Amino Acid Sequence and Figure 12 for the 323/A3 IgG_{4cys} variant Heavy Chain Amino Acid Sequence). See Figure 10 for schematic representation of the pEE18 plasmid encoding 323/A3 heavy and light chains.

2.2.2 Transfection and Selection of NSO Cells: see Example 6 above.

Example 8. Expression of the Humanized Antibody 323/A3(IgG_{2cys}) in NSO Cells

1. Purpose/Summary

The cDNAs encoding the humanized 323/A3(IgG_{2cys}) antibody heavy and light chains were genetically engineered into a single Celltech glutamine synthethase (GS) expression plamid, pEE18, and used to transfect murine NSO cells.

2. Materials and Methods

2.1 Materials as for Examples 6 and 7 above

2.2 Creation of 323/A3 (IgG_{2cys}) pEE18 Expression for Plasmid

The pEEE6 hcmv plasmid encoding full length humanized heavy chain DNA was digested with *Bam HI* and *Bgl II* to liberate 3.2 kb fragment that contained the DNA encoding the heavy chain under the transcriptional control of the major immediate early promoter of the human cytomegalovirus. This fragment was cloned into the *Bam II* site of pEE12 that contained the DNA encoding the humanized light chain (See Figure 13 for 323/A3(IgG_{2cys}) Kappa Light Chain Amino Acid Sequence and Figure 14 for the 323/A3(IgG_{2cys})Heavy Chain Amino Acid Sequence). See Figure 10 for schematic representation of the pEE18 plasmid encoding 323/A3 (IgG_{2cys}) heavy and light chains.

2.2.2 Transfection and Selection of NSO Cells - See Examples 6 and 7 above.

CLAIMS:

1. A combination of an anti-Ep-CAM antibody with a chemotherapeutic agent that is capable of arresting Ep-CAM antigen expressing cells in S or G_2/M.

2. A combination according to claim 1 wherein the Ep-CAM antibody is a 17.1A antibody.

3. A combination according to claim 2 wherein the Ep-CAM antibody is Panorex.

4. A combination according to any of the above claims wherein the chemotherapeutic agent is one or more agents selected from UFT, Capecitabine, CPT-II, Oxaliplatin, 5FU, 5FU continuous infusion, Paclitaxel, Docetaxel, Cyclophosphamide, Methotrexate, Doxorubicin, Navelbine (iv and oral), Epirubicin, Mitoxantrone, Raloxifen, Cisplatin, Mitomycin, Carboplatinum, Gemcitabine, Etoposide and Topotecan.

5. A combination according to claim 4, wherein the chemotherapeutic agent is CPT-II, 5FU (continuous infusion), Oxaliplatin, Capecitibine, UFT and Tomudex (Raloxifen).

6. A combination according to any of the above claims wherein the Ep-CAM expressing cells are cells of epithelial origin.

7. A combination according to any of the preceding claims wherein the Ep-CAM antigen expressing cells are tumour cells and their metastases.

8. A combination according to claim 7, wherein the Ep-CAM expressing tumour cells are adenocarcinoma cells and their metastases.

9. A combination according to claims 7 and 8, wherein the Ep-CAM expressing cells are prostate, lung, breast, gastric or colon originating cells or other tumours known to express the Ep-CAM antigen.

10. Use of an anti-Ep-CAM antibody in the manufacture of a medicament for use in anti-cancer therapy characterised in that a chemotherapeutic agent which is capable of arresting Ep-CAM antigen expressing cells in S or in G_2/M is co-administered to a patient with an anti-Ep-CAM antibody.

11. Use of an anti-Ep-CAM antibody according to claim 10 wherein the chemotherapeutic agent is administered prior to or simultaneously with the anti Ep-CAM antibody.

12. A method of increasing antibody binding of an anti-Ep-CAM antibody which comprises co-administering to a patient a chemotherapeutic agent capable of arresting cells in S or in G_2/M with an Ep-CAM antibody.

13. A method according to claim 11 which increases antibody binding between 2 to 10 fold compared to binding in the absence of said chemotherapeutic agent.

14. A method of treatment wherein a chemotherapeutic agent which is capable of arresting Ep-CAM antigen expressing cells in S or in G_2/M is co-administered to a patient with an anti-Ep-CAM antibody.

15. A pharmaceutical composition an anti-Ep-CAM antibody with a chemotherapeutic agent that is capable of arresting Ep-CAM antigen expressing cells in G_2/M.

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(54) Title: COMBINATION OF AN ANTI-EP-CAM ANTIBODY WITH A CHEMOTHERAPEUTIC AGENT

(57) Abstract: A combination of an anti-Ep-CAM antibody with a chemotherapeutic agent that is capable of arresting Ep-CAM antigen expressing cells in S or G_2/M.

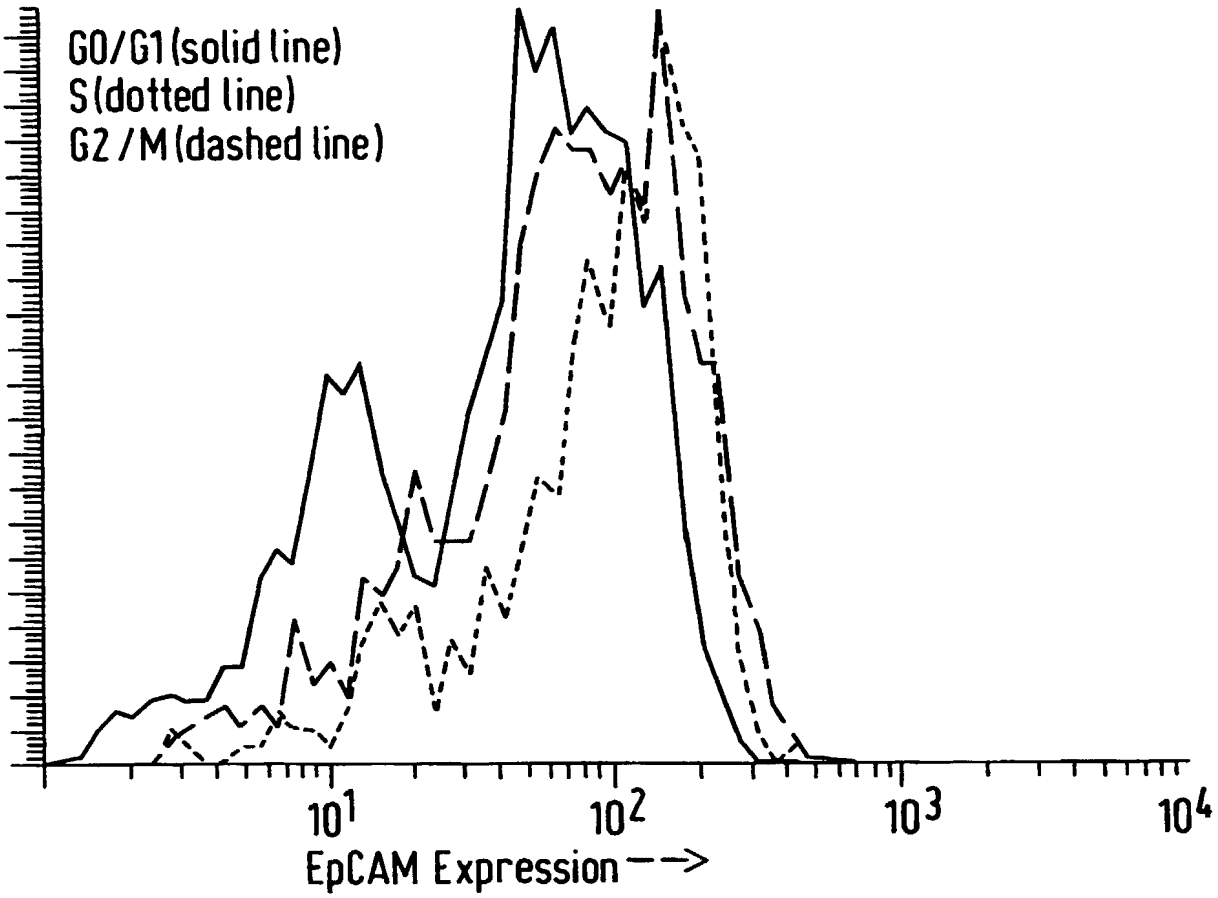

G0/G1 (solid line)
S (dotted line)
G2/M (dashed line)
10^1
10^2
10^3
10^4
EpCAM Expression →

FIG. 1

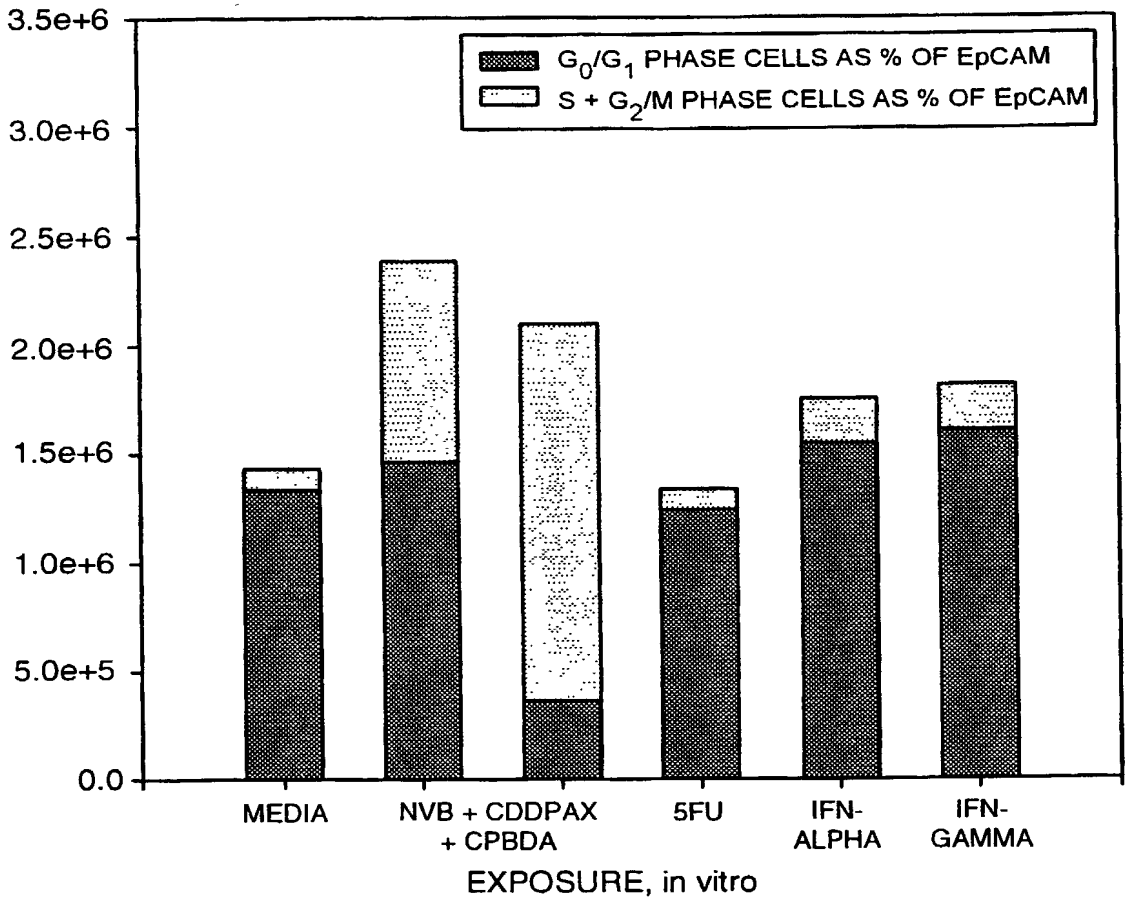

3.5e+6
3.0e+6
2.5e+6
2.0e+6
1.5e+6
1.0e+6
5.0e+5
0.0
G0/G1 PHASE CELLS AS % OF EpCAM
S + G2/M PHASE CELLS AS % OF EpCAM
MEDIA
NVB + CDDPAX + CPBDA
5FU
IFN-ALPHA
IFN-GAMMA
EXPOSURE, in vitro

FIG. 2

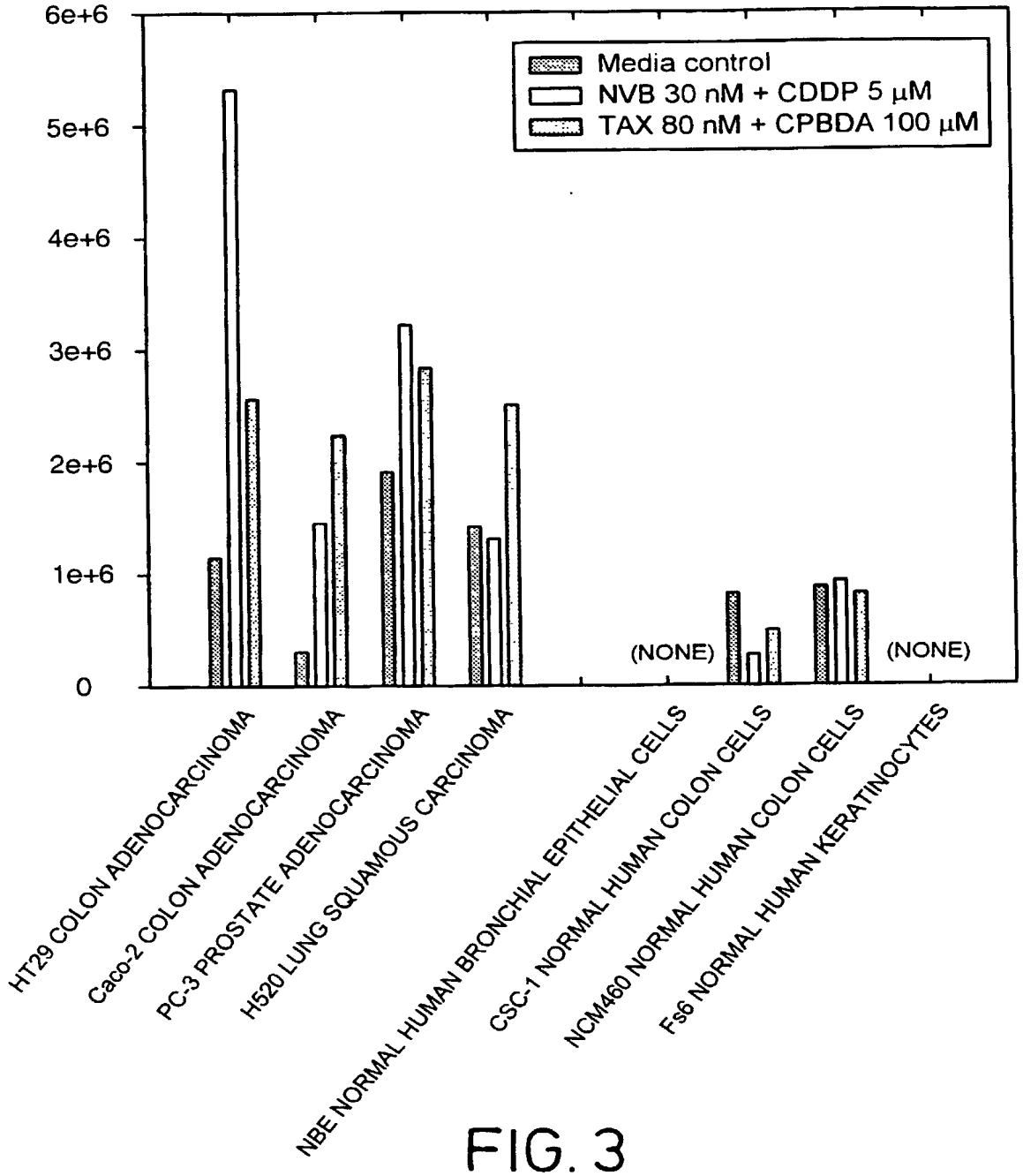

6e+6
5e+6
4e+6
3e+6
2e+6
1e+6
0
Media control
NVB 30 nM + CDDP 5 μM
TAX 80 nM + CPBDA 100 μM
(NONE)
(NONE)
HT29 COLON ADENOCARCINOMA
Caco-2 COLON ADENOCARCINOMA
PC-3 PROSTATE ADENOCARCINOMA
H520 LUNG SQUAMOUS CARCINOMA
NBE NORMAL HUMAN BRONCHIAL EPITHELIAL CELLS
CSC-1 NORMAL HUMAN COLON CELLS
NCM460 NORMAL HUMAN COLON CELLS
Fs6 NORMAL HUMAN KERATINOCYTES

FIG. 3

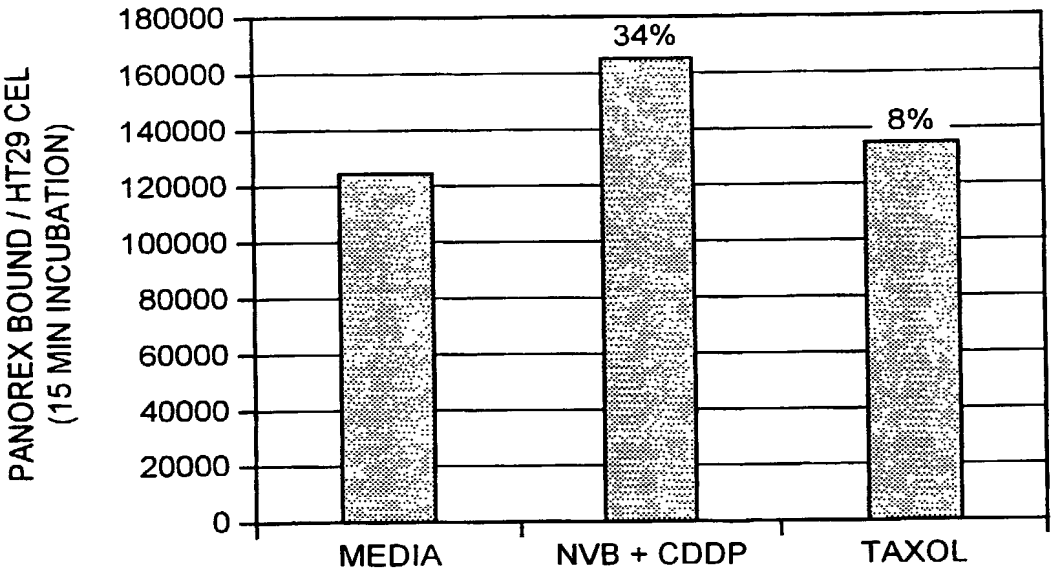

PANOREX BOUND / HT29 CEL
(15 MIN INCUBATION)
180000
160000
140000
120000
100000
80000
60000
40000
20000
0
34%
8%
MEDIA
NVB + CDDP
TAXOL

FIG. 3a

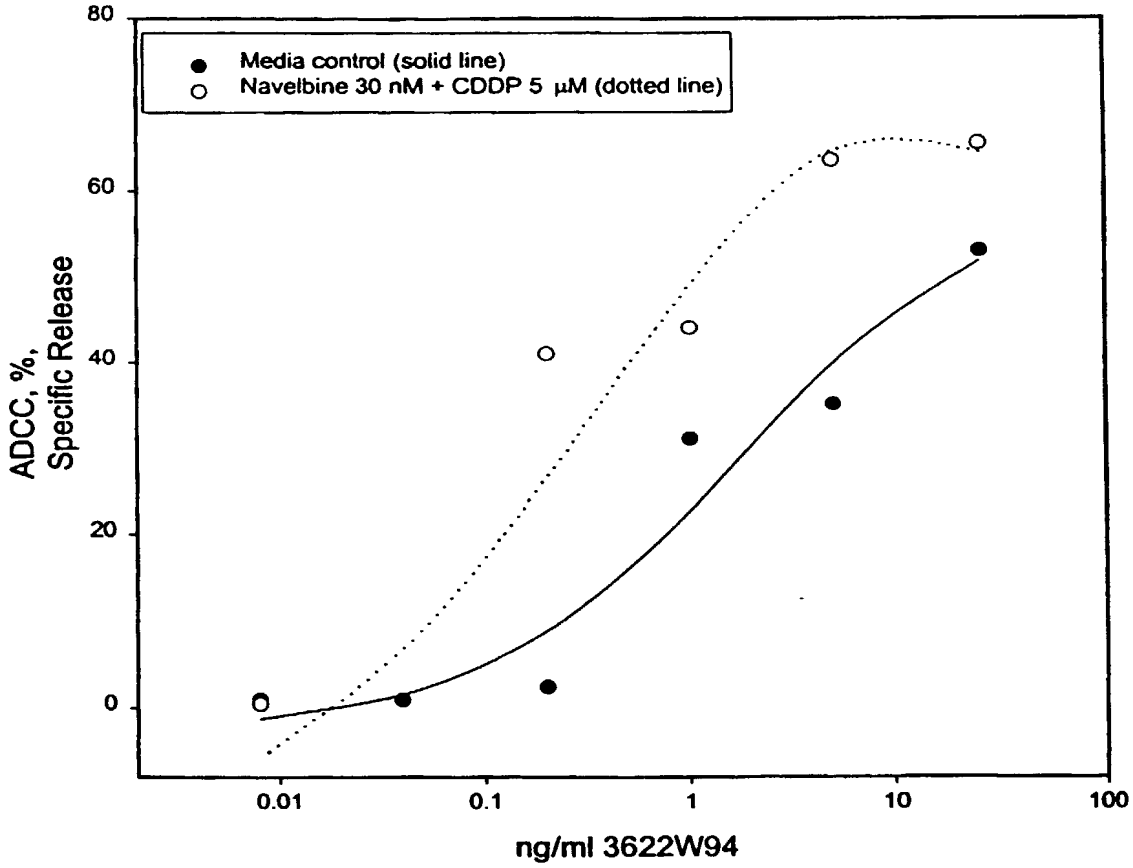

Media control (solid line)
Navelbine 30 nM + CDDP 5 µM (dotted line)
ADCC, %, Specific Release
80
60
40
20
0
0.01
0.1
1
10
100
ng/ml 3622W94

FIG. 4

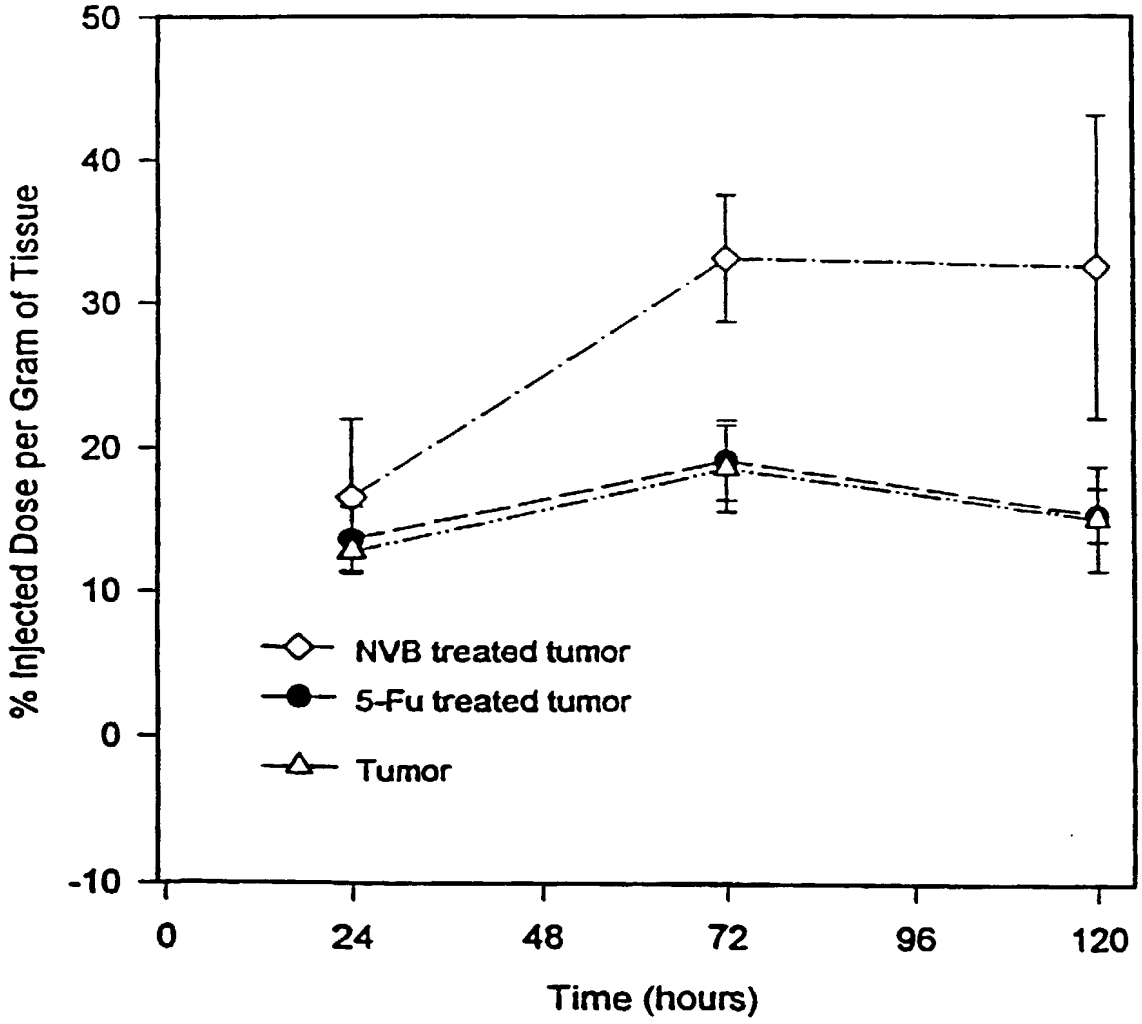

50
40
30
20
10
0
-10
% Injected Dose per Gram of Tissue
0
24
48
72
96
120
Time (hours)
NVB treated tumor
5-Fu treated tumor
Tumor

FIG.5

Humanised 323/A3 (IgG_1) Kappa Light Chain Amino Acid Sequence

The amino acid sequence of the humanized light chain of 323/A3 IgG_1, including leader peptide, is shown below.

1	MGWSCIILFL	VATATGVHSD	IVMTQSPLSL	PVTPGEPASI
41	SCRSSKNLLH	SNGITYLYWY	LQKPGQSPQL	LIYQMSNLAS
81	GVPDRFSSSG	SGTDFTLKIS	RVEAEDVGVY	YCAQNLEIPR
121	TFGQGTKVEI	KRTVAAPSVF	IFPPSDEQLK	SGTASVVCLL
161	NNFYPREAKV	QWKVDNALQS	GNSQESVTEQ	DSKDSTYSLS
201	STLTLSKADY	EKHKVYACEV	THQGLSSPVT	KSFNRGEC

FIG. 6

Humanised 323/A3 (IgG_1) Heavy Chain Amino Acid Sequence

The final amino acid sequence of the humanized heavy chain 323/A3 IgG_1, including leader peptide, is shown below.

1	MGWSCIILFL	VATATGVHSQ	VQLVQSGPEV	KKPGASVKVS
41	CKASGYTFTN	YGMNWVRQAP	GQGLEWMGWI	NTYTGEPTYG
81	EDFKGRFAFS	LDTSASTAYM	ELSSLRSEDT	AVYFCARFGN
121	YVDYWGQGSL	VTVSSASTKG	PSVFPLASS	KSTSGGTAAL
161	GCLVKDYFPE	PVTVSWNSGA	LTSGVHTFPA	VLQSSGLYSL
201	SSVVTVPSSS	LGTQTYICNV	NHKPSNTKVD	KKVEPKSCDK
241	THTCPPCPAP	ELLGGPSVFL	FPPKPKDTLM	ISRTPEVTCV
281	VVDVSHEDPE	VKFNWYVDGV	EVHNAKTKPR	EEQYNSTYRV
321	VSVLTVLHQD	WLNGKEYKCK	VSNKALPAPI	EKTISKAKGQ
361	PREPQVYTLP	PSRDELTKNQ	VSLTCLVKGF	YPSDIAVEWE
401	SNGQPENNYK	TTPPVLDSDG	SFFLYSKLTV	DKSRWQQGNV
441	FSCSVMHEAL	HNHYTQKSLS	LSPGK	

FIG. 7

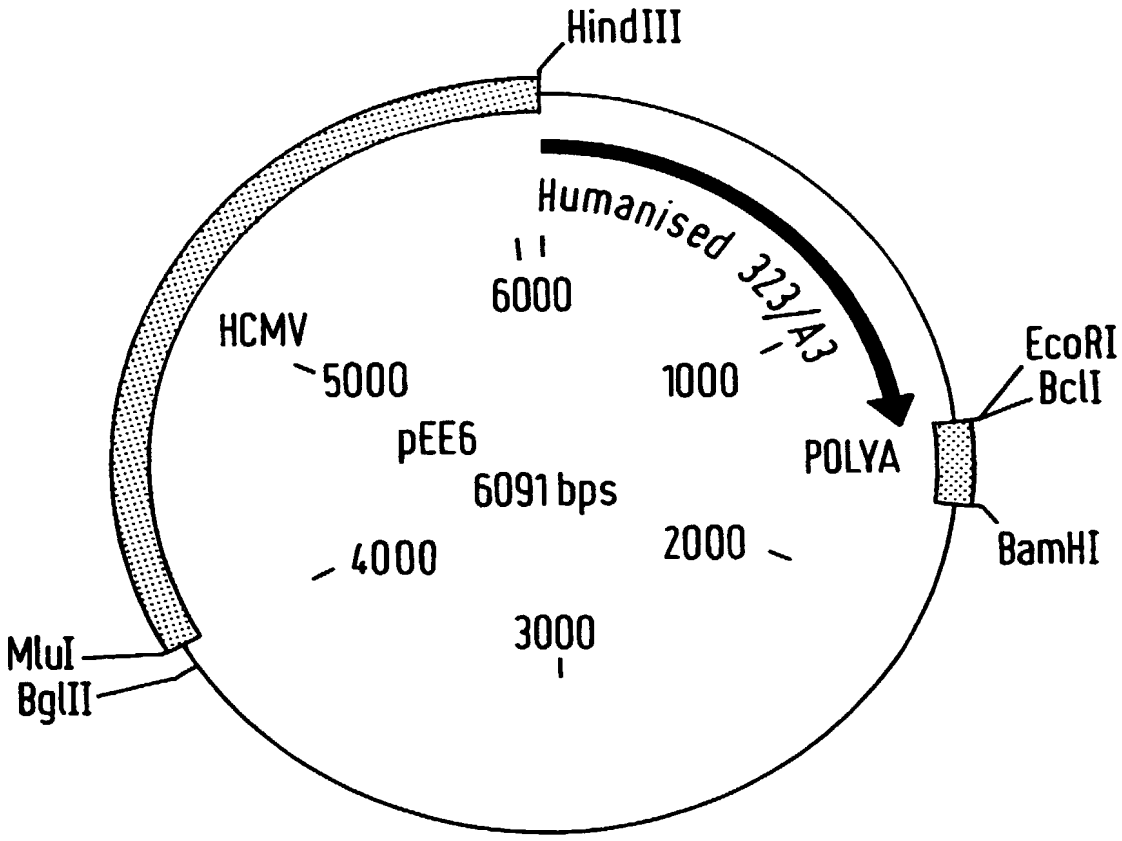

HindIII
Humanised 323/A3
6000
HCMV
5000
1000
EcoRI
BclI
pEE6
6091 bps
POLYA
4000
2000
BamHI
3000
MluI
BglII

FIG. 8

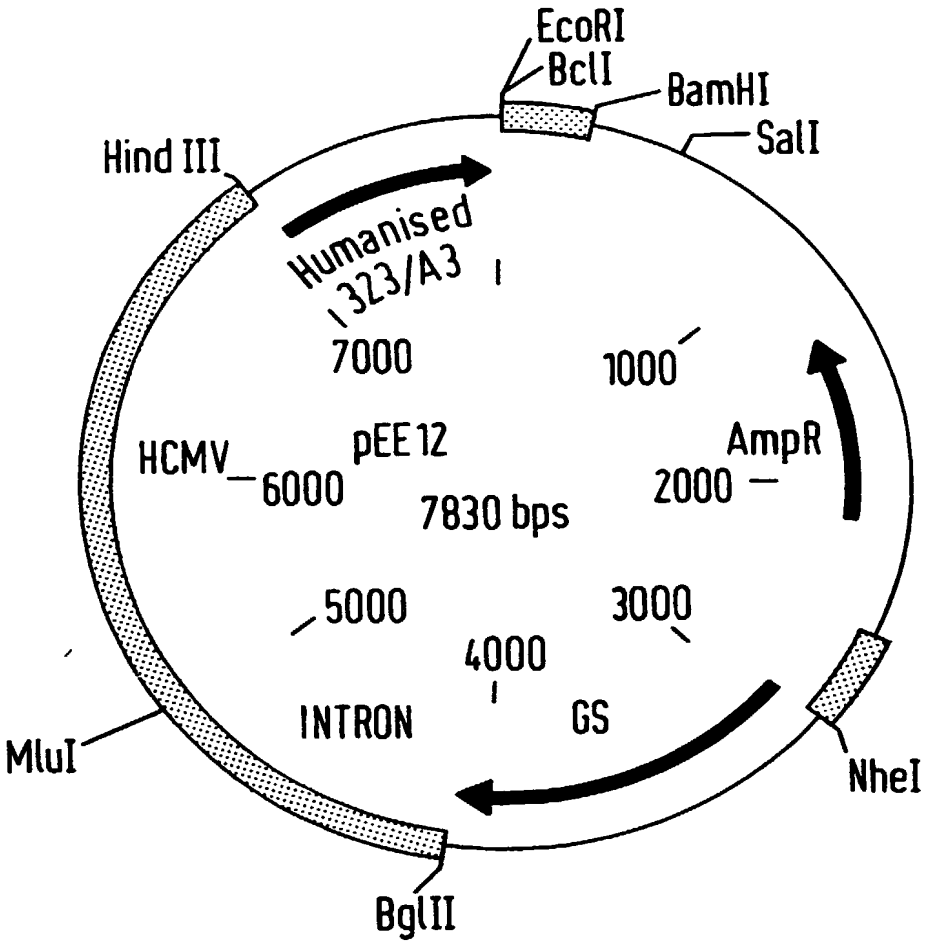

EcoRI
BclI
BamHI
SalI
Hind III
Humanised
323/A3
7000
1000
HCMV
6000
pEE12
7830 bps
AmpR
2000
5000
3000
4000
INTRON
GS
MluI
NheI
BglII

FIG. 9

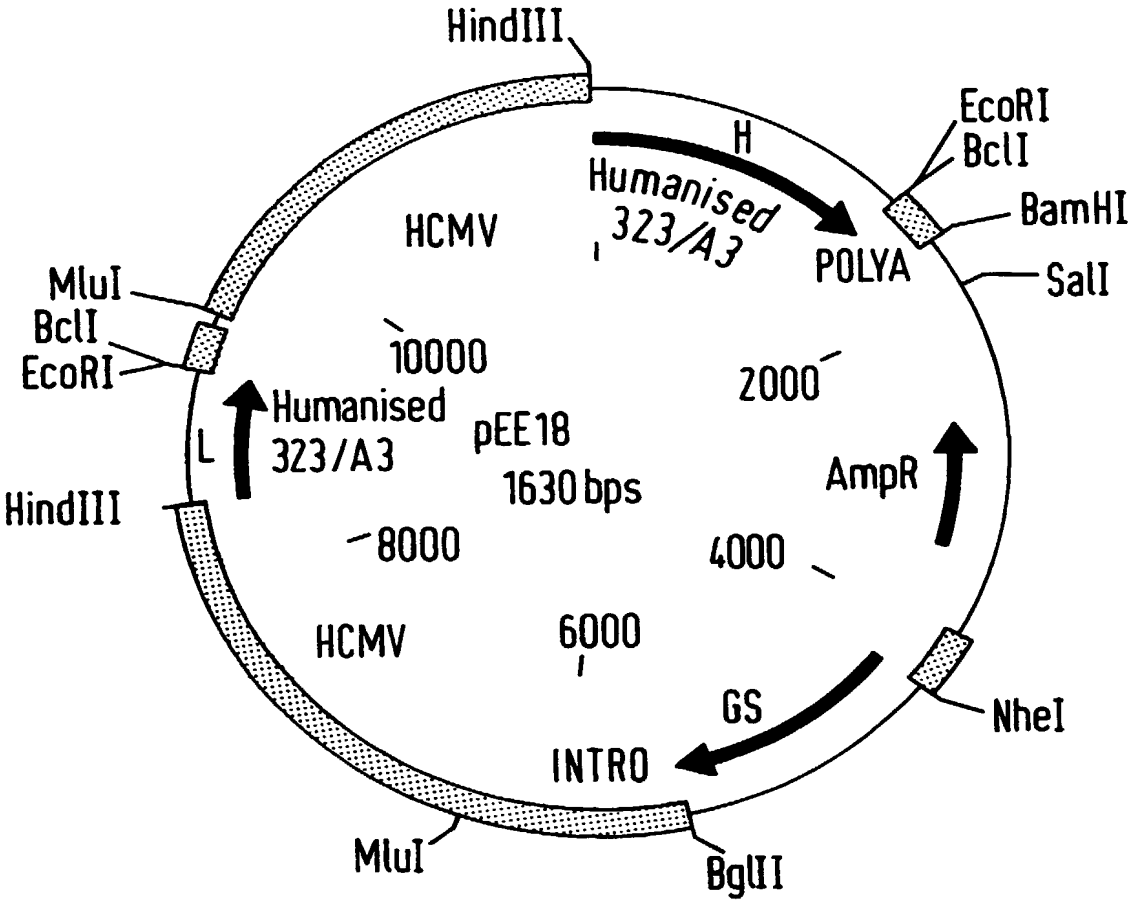

HindIII
EcoRI
BclI
BamHI
SalI
H
Humanised
323/A3
HCMV
POLYA
MluI
BclI
EcoRI
10000
2000
Humanised
323/A3
L
pEE18
1630 bps
AmpR
HindIII
8000
4000
HCMV
6000
GS
NheI
INTRO
MluI
BglII

FIG. 10

Humanised 323/A3 (IgG_{4cys}) Kappa Light Chain Amin Acid Sequence

The final amino acid sequence of the humanized light chain of 323/A3 IgG_4, including leader peptide, is shown below.

1	MGWSCIILFL	VATATGVHSD	IVMTQSPLSL	PVTPGEPASI
41	SCRSSKNLLH	SNGITYLYWY	LQKPGQSPQL	LIYQMSNLAS
81	GVPDRFSSSG	SGTDFTLKIS	RVEAEDVGVY	YCAQNLEIPR
121	TFGQGTKVEI	KRTVAAPSVF	IFPPSDEQLK	SGTASVVCLL
161	NNFYPREAKV	QWKVDNALQS	GNSQESVTEQ	DSKDSTYSLS
201	STLTLSKADY	EKHKVYACEV	THQGLSSPVT	KSFNRGEC

FIG. 11

Humanised 323/A3 (IgG_{4cys}) Heavy Chain Amino Acid Sequence

The final amino acid sequence of the humanized heavy chain 323/A3 IgG_4, including leader peptide, is shown below.

1	MGWSCIILFL	VATATGVHSQ	VQLVQSGPEV	KKPGASVKVS
41	CKASGYTFTN	YGMNWVRQAP	GQGLEWMGWI	NTYTGEPTYG
81	EDFKGRFAFS	LDTSASTAYM	ELSSLRSEDT	AVYFCARFGN
121	YVDYWGQGSL	VTVSSASTKG	PSVFPLAPCS	RSTSESTAAL
161	GCLVKDYFPE	PVTVSWNSGA	LTSGVHTFPA	VLQSSGLYSL
201	SSVVTVPSSS	LGTKTYTCNV	DHKPSNTKVD	KRVESKYGPP
241	CPPCPAPEFA	GAPSVFLFPP	KPKDTLMISR	TPEVTCVVVD
281	VSQEDPEVQF	NWYVDGVEVH	NAKTKPREEQ	FNSTYRVVSV
321	LTVLHQDWLN	GKAYKCKVSN	KGLPSSIEKT	ISKAKGQPRE
361	PQVYTLPPSQ	EEMTKNQVSL	TCLVKGFYPS	DIAVEWESNG
401	QPENNYKTTP	PVLDSDGSFF	LYSRLTVDKS	RWQEGNVFSC
441	SVMHEALHNH	YTQKSLCLSL	GK	

FIG. 12

Humanised 323/A3 (IgG_{2cys}) Kappa Light Chain Amino Acid Sequence

The final amino acid sequence of the humanized light chain of 323/A3 IgG_{2cys}, including leader peptide, is shown below.

1	MGWSCIILFL	VATATGVHSD	IVMTQSPLSL	PVTPGEPASI
41	SCRSSKNLLH	SNGITYLYWY	LQKPGQSPQL	LIYQMSNLAS
81	GVPDRFSSSG	SGTDFTLKIS	RVEAEDVGVY	YCAQNLEIPR
121	TFGQGTKVEI	KRTVAAPSVF	IFPPSDEQLK	SGTASVVCLL
161	NNFYPREAKV	QWKVDNALQS	GNSQESVTEQ	DSKDSTYSLS
201	STLTLSKADY	EKHKVYACEV	THQGLSSPVT	KSFNRGEC

FIG. 13

Humanised 323/A3 (IgG_{2cys}) Heavy Chain Amino Acid Sequence

The final amino acid sequence of the humanized heavy chain of 323/A3 IgG_{2cys}, including leader peptide, is shown below.

1	MGWSCIILFL	VATATGVHSQ	VQLVQSGPEV	KKPGASVKVS
41	CKASGYTFTN	YGMNWVRQAP	GQGLEWMGWI	NTYTGEPTYG
81	EDFKGRFAFS	LDTSASTAYM	ELSSLRSEDT	AVYFCARFGN
121	YVDYWGQGSL	VTVSSASTKG	PSVFPLAPCS	RSTSESTAAL
161	GCLVKDYFPE	PVTVSWNSGA	LTSGVHTFPA	VLQSSGLYSL
201	SSVVTVPSSN	FGTQTYTCNV	DHKPSNTKVD	KTVERKCCVE
241	CPPCPAPPVA	GPSVFLFPPK	PKDTLMISRT	PEVTCVVVDV
281	SHEDPEVQFN	WYVDGVEVHN	AKTKPREEQF	NSTFRVVSVL
321	TVVHQDWLNG	KEYKCKVSNK	GLPAPAIEKTI	SKTKGQPREP
361	QVYTLPPSRE	EMTKNQVSLT	CLVKGFYPSD	IAVEWESNGQ
401	PENNYKTTPP	MLDSDGSFFL	YSKLTVDKSR	WQQGNVFSCS
441	VMHEALHNHY	TQKSLCLSLG	K	

FIG. 14

Humanised 323/A3 (IgG_1) light chain DNA sequence
(also 323/A3 (IgG_{4cys} and IgG_{2cys} light chain cDNA sequence)

CGTAAGCTTC ACAGGACCTC ACC ATG GGA TGG AGC TGT ATC ATC CTC TTC TTG
GCATTCGAAG TGTCCTGGAG TGG TAC CCT ACC TCG ACA TAG TAG GAG AAG AAC
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu>

GTA GCA ACA GCT ACA GGT GTC CAC TCC GAT ATT GTG ATG ACT CAG TCT
CAT CGT TGT CGA TGT CCA CAG GTG AGG CTA TAA CAC TAC TGA GTC AGA
Val Ala Thr Ala Thr Gly Val His Ser>
Asp Ile Val Met Thr Gln Ser>

CCA CTC TCC CTG CCC GTC ACC CCT GGA GAG CCG GCC TCC ATC TCC TGT
GGT GAG AGG GAC GGG CAG TGG GGA CCT CTC GGC CGG AGG TAG AGG ACA
Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys>

AGG TCT AGT AAG AAT CTC CTG CAT AGT AAT GGC ATC ACT TAT TTG TAT
TCC AGA TCA TTC TTA GAG GAC GTA TCA TTA CCG TAG TGA ATA AAC ATA
Arg Ser Ser Lys Asn Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr>

TGG TAC CTG CAG AAG CCA GGG CAG TCT CCA CAG CTC CTG ATC TAT CAG
ACC ATG GAC GTC TTC GGT CCC GTC AGA GGT GTC GAG GAC TAG ATA GTC
Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln>

ATG TCC AAC CTT GCC TCA GGG GTC CCT GAC AGG TTC AGT AGC AGT GGA
TAC AGG TTG GAA CGG AGT CCC CAG GGA CTG TCC AAG TCA TCG TCA CCT
Met Ser Asn Leu Ala Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly>

TCA GGC ACA GAT TTT ACA CTG AAA ATC AGC AGA GTG GAG GCT GAG GAT
AGT CCG TGT CTA AAA TGT GAC TTT TAG TCG TCT CAC CTC CGA CTC CTA
Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp>

GTT GGG GTT TAT TAC TGT GCT CAA AAT CTA GAG ATT CCT CGG ACG TTC
CAA CCC CAA ATA ATG ACA CGA GTT TTA GAT CTC TAA GGA GCC TGC AAG
Val Gly Val Tyr Tyr Cys Ala Gln Asn Leu Glu Ile Pro Arg Thr Phe>

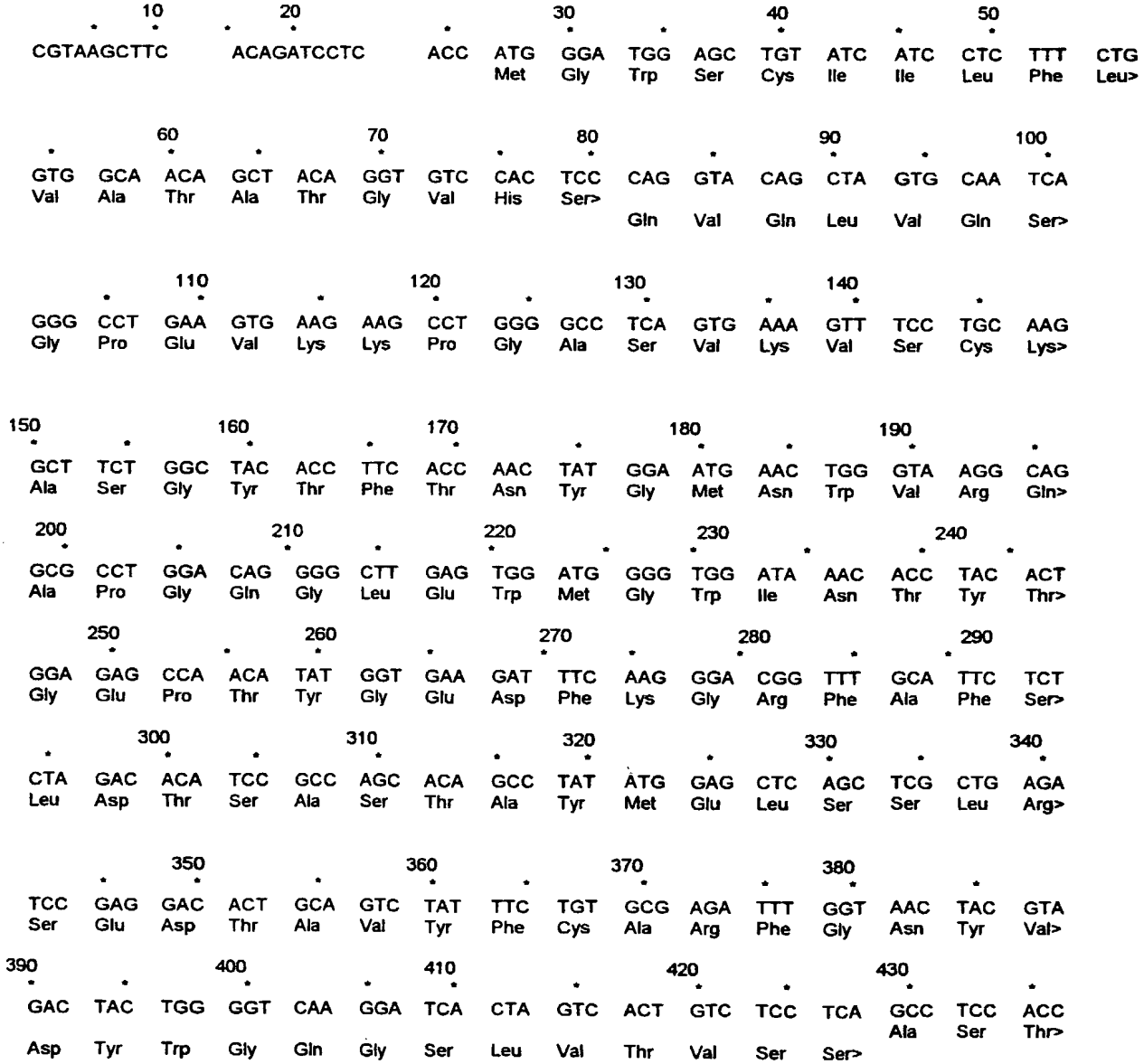
FIG. 15

```
390                 400                 410                 420                 430
GGC CAA GGG ACC AAG GTG GAG ATC AAA CGT ACG GTG GCT GCA CCA TCT
CCG GTT CCC TGG TTC CAC CTC TAG TTT GCA TGC CAC CGA CGT GGT AGA
Gly Gln Gly Thr Lys Val Glu Ile Lys Arg>
                                        Thr Val Ala Ala Pro Ser>

440                 450                 460                 470                 480
GTC TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA ACT GCC
CAG AAG TAG AAG GGC GGT AGA CTA CTC GTC AAC TTT AGA CCT TGA CGG

Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala>

        490                 500                 510                 520                 530
TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA GAG GCC AAA GTA
AGA CAA CAC ACG GAC GAC TTA TTG AAG ATA GGG TCT CTC CGG TTT CAT
Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val>

            540                 550                 560                 570                 580
CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG GAG AGT
GTC ACC TTC CAC CTA TTG CGG GAG GTT AGC CCA TTG AGG GTC CTC TCA
Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser>

            590                 600                 610                 620
GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC
CAG TGT CTC GTC CTG TCG TTC CTG TCG TGG ATG TCG GAG TCG TCG TGG
Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr>

630                 640                 650                 660                 670
CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC
GAC TGC GAC TCG TTT CGT CTG ATG CTC TTT GTG TTT CAG ATG CGG ACG
Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys>

680                 690                 700                 710                 720
GAA GTC ACC CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG AGC TTC AAC
CTT CAG TGG GTA GTC CCG GAC TCG AGC GGG CAG TGT TTC TCG AAG TTG
Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn>

        730                 740
AGG GGA GAG TGT TAG
TCC CCT CTC ACA ATC
Arg Gly Glu Cys ***>
```

FIG. 15 cont.

FIG. 16

Humanised 323/A3 (IgG_1) heavy chain DNA sequence



```
440                 450                 460                 470                 480
AAG GGC CCA TCG GTC TTC CCC CTG GCA CCC TCC TCC AAG AGC ACC TCT
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser>

      490                 500                 510                 520                 530
GGG GGC ACA GCG GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu>

            540                 550                 560                 570                 580
CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His>

      590                 600                 610                 620
ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser>

630                 640                 650                 660                 670
GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACC CAG ACC TAC ATC TGC
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys>

680                 690                 700                 710                 720
AAC GTG AAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AAA GTT GAG
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu>

      730                 740                 750                 760                 770
CCC AAA TCT TGT GAC AAA ACT CAC ACA TGC CCA CCG TGC CCA GCA CCT
Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro>

            780                 790                 800                 810                 820
GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys>

      830                 840                 850                 860
GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACA TGC GTG GTG GTG
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val>

870                 880                 890                 900                 910
GAC GTG AGC CAC GAA GAC CCT GAG GTC AAG TTC AAC TGG TAC GTG GAC
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp>
```

FIG. 16 cont.

```
920                 930                 940                 950                 960
GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TAC
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr>

970                 980                 990                 1000                1010
AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp>

1020                1030                1040                1050                1060
TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA GCC CTC
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu>

1070                1080                1090                1100
CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg>

1110                1120                1130                1140                1150
GAA CCA CAG GTG TAC ACC CTG CCC CCA TCC CGG GAT GAG CTG ACC AAG
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys>

1160                1170                1180                1190                1200
AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp>

1210                1220                1230                1240                1250
ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys>

1260                1270                1280                1290                1300
ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser>

1310                1320                1330                1340
AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser>

1350                1360                1370                1380                1390
TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser>

1400                1410
CTC TCC CTG TCT CCG GGT AAA
Leu Ser Leu Ser Pro Gly Lys>
```

FIG. 16 cont.

FIG. 17.

Humanised 323/A3 (IgG_{4cys}) heavy chain cDNA sequence)

```
                10                  20                  30                  40                  50
                .                   .                   .                   .                   .
CGTAAGCTTC      ACAGATCCTC      ACC ATG GGA TGG AGC TGT ATC ATC CTC TTT CTG
                                    Met Gly Trp Ser Cys Ile Ile Leu Phe Leu>

            60                  70                  80                  90                  100
            .                   .                   .                   .                   .
GTG GCA ACA GCT ACA GGT GTC CAC TCC CAG GTA CAG CTA GTG CAA TCA
Val Ala Thr Ala Thr Gly Val His Ser Xxx>
                                    Gln Val Gln Leu Val Gln Ser>

        110                 120                 130                 140
        .                   .                   .                   .
GGG CCT GAA GTG AAG AAG CCT GGG GCC TCA GTG AAA GTT TCC TGC AAG
Gly Pro Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys>

150             160                 170                 180                 190
.               .                   .                   .                   .
GCT TCT GGC TAC ACC TTC ACC AAC TAT GGA ATG AAC TGG GTA AGG CAG
Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Arg Gln>

200                 210                 220                 230                 240
.                   .                   .                   .                   .
GCG CCT GGA CAG GGG CTT GAG TGG ATG GGG TGG ATA AAC ACC TAC ACT
Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr>

    250                 260                 270                 280                 290
    .                   .                   .                   .                   .
GGA GAG CCA ACA TAT GGT GAA GAT TTC AAG GGA CGG TTT GCA TTC TCT
Gly Glu Pro Thr Tyr Gly Glu Asp Phe Lys Gly Arg Phe Ala Phe Ser>

        300                 310                 320                 330                 340
        .                   .                   .                   .                   .
CTA GAC ACA TCC GCC AGC ACA GCC TAT ATG GAG CTC AGC TCG CTG AGA
Leu Asp Thr Ser Ala Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg>

            350                 360                 370                 380
            .                   .                   .                   .
TCC GAG GAC ACT GCA GTC TAT TTC TGT GCG AGA TTT GGT AAC TAC GTA
Ser Glu Asp Thr Ala Val Tyr Phe Cys Ala Arg Phe Gly Asn Tyr Val>

390             400                 410                 420                 430
.               .                   .                   .                   .
GAC TAC TGG GGT CAA GGA TCA CTA GTC ACT GTC TCC TCA GCT TCC ACC
Asp Tyr Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser>  Ala Ser Thr>

440                 450                 460                 470                 480
.                   .                   .                   .                   .
AAG GGC CCA TCC GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser>
```

```
      490         500         510         520         530
GAG AGC ACA GCC GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA
Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu>

      540         550         560         570         580
CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His>

      590         600         610         620
ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser>

630         640         650         660         670
GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACG AAG ACC TAC ACC TGC
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys>

680         690         700         710         720
AAC GTA GAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA GTT GAG
Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu>

      730         740         750         760         770
TCC AAA TAT GGT CCC CCA TGC CCA CCG TGC CCT GCA CCT GAG TTC GCG
Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Ala>

      780         790         800         810         820
GGG GCA CCA TCA GTC TTC CTG TTC CCC CCA AAA CCC AAG GAC ACT CTC
Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu>

      830         840         850         860
ATG ATC TCC CGG ACC CCT GAG GTC ACG TGC GTG GTG GTG GAC GTG AGC
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser>

870         880         890         900         910
CAG GAA GAC CCC GAG GTC CAG TTC AAC TGG TAC GTG GAT GGC GTG GAG
Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu>

      920         930         940         950         960
GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TTC AAC AGC ACG
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr>
```

FIG. 17cont.

```
          970                 980                 990                1000                1010
TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG ACC
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn>

     1020                1030                1040                1050                1060
GGC AAG GCG TAC AAG TGC AAG GTC TCC AAC AAA GGC CTC CCG TCC TCC
Gly Lys Ala Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser>

          1070                1080                1090                1100
ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAG CCA CAG
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln>

1110                1120                1130                1140                1150
GTG TAC ACC CTG CCC CCA TCC CAG GAG GAG ATG ACC AAG AAC CAG GTC
Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val>

     1160                1170                1180                1190                1200
AGC CTG ACC TGC CTG GTC AAA GGC TTC TAC CCC AGC GAC ATC GCC GTG
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val>

          1210                1220                1230                1240                1250
GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro>

     1260                1270                1280                1290                1300
CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AGG CTA ACC
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr>

          1310                1320                1330                1340
GTG GAC AAG AGC AGG TGG CAG GAG GGG AAT GTC TTC TCA TGC TCC GTG
Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val>

1350                1360                1370                1380                1390
ATG CAT GAG GCT CTG CAC AAC CAC TAC ACA CAG AAG AGC CTC TGC CTG
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Cys Leu>

     1400                1410
TCT CTG GGT AAA T   GAGAATTC
Ser Leu Gly Lys>
```

FIG. 17cont.

FIG. 18.

Humanised 323/A3 (IgG_{2cys}) heavy chain cDNA sequence

```
          10         20         30         40         50         60
ATGGATTGGC TGTGGAACTT GCTATTCCTG ATGGCAGCTG CCCAAAGTAT CCAAGCA CAG
TACCTAACCG ACACCTTGAA CGATAAGGAC TACCGTCGAC GGGTTTCATA GGTTCGT GTC
                                                              Gln>

              70         80         90        100
ATC CAG TTG GTG CAG TCT GGA CCT GAA CTG AAG AAG CCT GGA GAG ACA
TAG GTC AAC CAC GTC AGA CCT GGA CTT GAC TTC TTC GGA CCT CTC TGT
Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu Thr>

110        120        130        140        150
GTC AAG ATC TCC TGC AAG GCT TCT GGA TAT ACC TTC ACA AAC TAT GGA
CAG TTC TAG AGG ACG TTC CGA AGA CCT ATA TGG AAG TGT TTG ATA CCT
Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly>

    160        170        180        190        200
ATG AAC TGG GTG AGG CAG GCT TCA GGA GAG GGT TTA AAG TGG ATG GGC
TAC TTG ACC CAC TCC GTC CGA AGT CCT CTC CCA AAT TTC ACC TAC CCG
Met Asn Trp Val Arg Gln Ala Ser Gly Glu Gly Leu Lys Trp Met Gly>

        210        220        230        240        250
TGG ATA AAC ACC TAC ACT GGA GAG CCA ACA TAT GGT GAA GAT TTC AAG
ACC TAT TTG TGG ATG TGA CCT CTC GGT TGT ATA CCA CTT CTA AAG TTC
Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Gly Glu Asp Phe Lys>

            260        270        280        290        300
GGA CGG TTT GCC TTC TCT TTG GAA ACC TCT GCC AGC ACT GCC TAT TTG
CCT GCC AAA CGG AAG AGA AAC CTT TGG AGA CGG TCG TGA CGG ATA AAC
Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr Leu>

                310        320        330        340
CAG ATC AAC AAC CTC AAA AAT GAA GAC ACG GCT ACA TAT TTC TGT GCA
GTC TAG TTG TTG GAG TTT TTA CTT CTG TGC CGA TGT ATA AAG ACA CGT
Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys Ala>

350        360        370        380        390
AGA TTT GGT AAC TAC GTA GAC TAC TGG GGC CAA GGC ACC ACT CTC ACA
TCT AAA CCA TTG ATG CAT CTG ATG ACC CCG GTT CCG TGG TGA GAG TGT
Arg Phe Gly Asn Tyr Val Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr>

    400        410        420        430        440
GTC TCC TCA GCC TCC ACC AAG GGC CCA TCG GTC TTC CCC CTG GCG CCC
CAG AGG AGT CGG AGG TGG TTC CCG GGT AGC CAG AAG GGG GAC CGC GGG
Val Ser Ser>
            Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro>
```

```
      450                 460                 470                 480                 490
      .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
TGC   TCC   AGG   AGC   ACC   TCC   GAG   AGC   ACA   GCG   GCC   CTG   GGC   TGC   CTG   GTC
ACG   AGG   TCC   TCG   TGG   AGG   CTC   TCG   TGT   CGC   CGG   GAC   CCG   ACG   GAC   CAG
Cys   Ser   Arg   Ser   Thr   Ser   Glu   Ser   Thr   Ala   Ala   Leu   Gly   Cys   Leu   Val>

      500                 510                 520                 530                 540
      .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
AAG   GAC   TAC   TTC   CCC   GAA   CCG   GTG   ACG   GTG   TCG   TGG   AAC   TCA   GGC   GCT
TTC   CTG   ATG   AAG   GGG   CTT   GGC   CAC   TGC   CAC   AGC   ACC   TTG   AGT   CCG   CGA
Lys   Asp   Tyr   Phe   Pro   Glu   Pro   Val   Thr   Val   Ser   Trp   Asn   Ser   Gly   Ala>

      550                 560                 570                 580
      .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
CTG   ACC   AGC   GGC   GTG   CAC   ACC   TTC   CCA   GCT   GTC   CTA   CAG   TCC   TCA   GGA
GAC   TGG   TCG   CCG   CAC   GTG   TGG   AAG   GGT   CGA   CAG   GAT   GTC   AGG   AGT   CCT
Leu   Thr   Ser   Gly   Val   His   Thr   Phe   Pro   Ala   Val   Leu   Gln   Ser   Ser   Gly>

590                 600                 610                 620                 630
      .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
CTC   TAC   TCC   CTC   AGC   AGC   GTG   GTG   ACC   GTG   CCC   TCC   AGC   AAC   TTC   GGC
GAG   ATG   AGG   GAG   TCG   TCG   CAC   CAC   TGG   CAC   GGG   AGG   TCG   TTG   AAG   CCG
Leu   Tyr   Ser   Leu   Ser   Ser   Val   Val   Thr   Val   Pro   Ser   Ser   Asn   Phe   Gly>

      640                 650                 660                 670                 680
      .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
ACC   CAG   ACC   TAC   ACC   TGC   AAC   GTA   GAT   CAC   AAG   CCC   AGC   AAC   ACC   AAG
TGG   GTC   TGG   ATG   TGG   ACG   TTG   CAT   CTA   GTG   TTC   GGG   TCG   TTG   TGG   TTC
Thr   Gln   Thr   Tyr   Thr   Cys   Asn   Val   Asp   His   Lys   Pro   Ser   Asn   Thr   Lys>

      690                 700                 710                 720                 730
      .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
GTG   GAC   AAG   ACA   GTT   GAG   CGC   AAA   TGT   TGT   GTC   GAG   TGC   CCA   CCG   TGC
CAC   CTG   TTC   TGT   CAA   CTC   GCG   TTT   ACA   ACA   CAG   CTC   ACG   GGT   GGC   ACG
Val   Asp   Lys   Thr   Val   Glu   Arg   Lys   Cys   Cys   Val   Glu   Cys   Pro   Pro   Cys>

      740                 750                 760                 770                 780
      .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
CCA   GCA   CCA   CCT   GTG   GCA   GGA   CCG   TCA   GTC   TTC   CTC   TTC   CCC   CCA   AAA
GGT   CGT   GGT   GGA   CAC   CGT   CCT   GGC   AGT   CAG   AAG   GAG   AAG   GGG   GGT   TTT
Pro   Ala   Pro   Pro   Val   Ala   Gly   Pro   Ser   Val   Phe   Leu   Phe   Pro   Pro   Lys>

      790                 800                 810                 820
      .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
CCC   AAG   GAC   ACC   CTC   ATG   ATC   TCC   CGG   ACC   CCT   GAG   GTC   ACG   TGC   GTG
GGG   TTC   CTG   TGG   GAG   TAC   TAG   AGG   GCC   TGG   GGA   CTC   CAG   TGC   ACG   CAC
Pro   Lys   Asp   Thr   Leu   Met   Ile   Ser   Arg   Thr   Pro   Glu   Val   Thr   Cys   Val>

830                 840                 850                 860                 870
      .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
GTG   GTG   GAC   GTG   AGC   CAC   GAA   GAC   CCC   GAG   GTC   CAG   TTC   AAC   TGG   TAC
CAC   CAC   CTG   CAC   TCG   GTG   CTT   CTG   GGG   CTC   CAG   GTC   AAG   TTG   ACC   ATG
Val   Val   Asp   Val   Ser   His   Glu   Asp   Pro   Glu   Val   Gln   Phe   Asn   Trp   Tyr>
```

FIG. 18cont.

```
      880                 890                 900                 910                 920
      .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
GTG   GAC   GGC   GTG   GAG   GTG   CAT   AAT   GCC   AAG   ACA   AAG   CCA   CGG   GAG   GAG
CAC   CTG   CCG   CAC   CTC   CAC   GTA   TTA   CGG   TTC   TGT   TTC   GGT   GCC   CTC   CTC
Val   Asp   Gly   Val   Glu   Val   His   Asn   Ala   Lys   Thr   Lys   Pro   Arg   Glu   Glu>

      930                 940                 950                 960                 970
   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
CAG   TTC   AAC   AGC   ACG   TTC   CGT   GTG   GTC   AGC   GTC   CTC   ACC   GTT   GTG   CAC
GTC   AAG   TTG   TCG   TGC   AAG   GCA   CAC   CAG   TCG   CAG   GAG   TGG   CAA   CAC   GTG
Gln   Phe   Asn   Ser   Thr   Phe   Arg   Val   Val   Ser   Val   Leu   Thr   Val   Val   His>

         980                 990                1000                1010                1020
   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
CAG   GAC   TGG   CTG   AAC   GGC   AAG   GAG   TAC   AAG   TGC   AAG   GTC   TCC   AAC   AAA
GTC   CTG   ACC   GAC   TTG   CCG   TTC   CTC   ATG   TTC   ACG   TTC   CAG   AGG   TTG   TTT
Gln   Asp   Trp   Leu   Asn   Gly   Lys   Glu   Tyr   Lys   Cys   Lys   Val   Ser   Asn   Lys>

            1030                1040                1050                1060
   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
GGC   CTC   CCA   GCC   CCC   ATC   GAG   AAA   ACC   ATC   TCC   AAA   ACC   AAA   GGG   CAG
CCG   GAG   GGT   CGG   GGG   TAG   CTC   TTT   TGG   TAG   AGG   TTT   TGG   TTT   CCC   GTC
Gly   Leu   Pro   Ala   Pro   Ile   Glu   Lys   Thr   Ile   Ser   Lys   Thr   Lys   Gly   Gln>

1070                1080                1090                1100                1110
   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
CCC   CGA   GAA   CCA   CAG   GTG   TAC   ACC   CTG   CCC   CCA   TCC   CGG   GAG   GAG   ATG
GGG   GCT   CTT   GGT   GTC   CAC   ATG   TGG   GAC   GGG   GGT   AGG   GCC   CTC   CTC   TAC
Pro   Arg   Glu   Pro   Gln   Val   Tyr   Thr   Leu   Pro   Pro   Ser   Arg   Glu   Glu   Met>

      1120                1130                1140                1150                1160
   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
ACC   AAG   AAC   CAG   GTC   AGC   CTG   ACC   TGC   CTG   GTC   AAA   GGC   TTC   TAC   CCC
TGG   TTC   TTG   GTC   CAG   TCG   GAC   TGG   ACG   GAC   CAG   TTT   CCG   AAG   ATG   GGG
Thr   Lys   Asn   Gln   Val   Ser   Leu   Thr   Cys   Leu   Val   Lys   Gly   Phe   Tyr   Pro>

         1170                1180                1190                1200                1210
   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
AGC   GAC   ATC   GCC   GTG   GAG   TGG   GAG   AGC   AAT   GGG   CAG   CCG   GAG   AAC   AAC
TCG   CTG   TAG   CGG   CAC   CTC   ACC   CTC   TCG   TTA   CCC   GTC   GGC   CTC   TTG   TTG
Ser   Asp   Ile   Ala   Val   Glu   Trp   Glu   Ser   Asn   Gly   Gln   Pro   Glu   Asn   Asn>

            1220                1230                1240                1250                1260
   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .   .
TAC   AAG   ACC   ACA   CCT   CCC   ATG   CTG   GAC   TCC   GAC   GGC   TCC   TTC   TTC   CTC
ATG   TTC   TGG   TGT   GGA   GGG   TAC   GAC   CTG   AGG   CTG   CCG   AGG   AAG   AAG   GAG
Tyr   Lys   Thr   Thr   Pro   Pro   Met   Leu   Asp   Ser   Asp   Gly   Ser   Phe   Phe   Leu>
```

FIG. 18 cont.

```
                1270                1280                1290                1300
                 .                   .                   .                   .
TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC
ATG TCG TTC GAG TGG CAC CTG TTC TCG TCC ACC GTC GTC CCC TTG CAG
Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val>

1310            1320                1330                1340                1350
 .               .                   .                   .                   .
TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACA CAG
AAG AGT ACG AGG CAC TAC GTA CTC CGA GAC GTG TTG GTG ATG TGT GTC
Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln>

   1360                1370                1380                1390
    .                   .                   .                   .
AAG AGC CTC TGC CTG TCT CTG GGT AAA TGAGAAT TC
TTC TCG GAG ACG GAC AGA GAC CCA TTT ACTCTTA AG
Lys Ser Leu Cys Leu Ser Leu Gly Lys>
```

FIG. 18cont.

SEQUENCE LISTING

<110> Glaxo Group Limited
Knick, Vincent C
Stimmel, Julie B
Thurmond, Linda M

<120> Antibody combination

<130> PU3513

<140>
<141>

<150> GB 9816280.3
<151> 1998-07-27

<160> 16

<170> PatentIn Ver. 2.1

<210> 1
<211> 740
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (24)..(740)

<220>
<223> Description of Artificial Sequence: Synthetic sequence

<400> 1
cgtaagcttc acaggacctc acc atg gga tgg agc tgt atc atc ctc ttc ttg 53
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu
1 5 10

```
gta gca aca gct aca ggt gtc cac tcc gat att gtg atg act cag tct   101
Val Ala Thr Ala Thr Gly Val His Ser Asp Ile Val Met Thr Gln Ser
                15                  20                  25

cca ctc tcc ctg ccc gtc acc cct gga gag ccg gcc tcc atc tcc tgt   149
Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys
            30                  35                  40

agg tct agt aag aat ctc ctg cat agt aat ggc atc act tat ttg tat   197
Arg Ser Ser Lys Asn Leu Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr
        45                  50                  55

tgg tac ctg cag aag cca ggg cag tct cca cag ctc ctg atc tat cag   245
Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln
    60                  65                  70

atg tcc aac ctt gcc tca ggg gtc cct gac agg ttc agt agc agt gga   293
Met Ser Asn Leu Ala Ser Gly Val Pro Asp Arg Phe Ser Ser Ser Gly
75                  80                  85                  90

tca ggc aca gat ttt aca ctg aaa atc agc aga gtg gag gct gag gat   341
Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp
                95                  100                 105

gtt ggg gtt tat tac tgt gct caa aat cta gag att cct cgg acg ttc   389
Val Gly Val Tyr Tyr Cys Ala Gln Asn Leu Glu Ile Pro Arg Thr Phe
            110                 115                 120

ggc caa ggg acc aag gtg gag atc aaa cgt acg gtg gct gca cca tct   437
Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val Ala Ala Pro Ser
        125                 130                 135

gtc ttc atc ttc ccg cca tct gat gag cag ttg aaa tct gga act gcc   485
Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala
    140                 145                 150
```

```
tct gtt gtg tgc ctg ctg aat aac ttc tat ccc aga gag gcc aaa gta    533
Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val
155                 160                 165                 170

cag tgg aag gtg gat aac gcc ctc caa tcg ggt aac tcc cag gag agt    581
Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser
                175                 180                 185

gtc aca gag cag gac agc aag gac agc acc tac agc ctc agc agc acc    629
Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr
            190                 195                 200

ctg acg ctg agc aaa gca gac tac gag aaa cac aaa gtc tac gcc tgc    677
Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys
        205                 210                 215

gaa gtc acc cat cag ggc ctg agc tcg ccc gtc aca aag agc ttc aac    725
Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn
    220                 225                 230

agg gga gag tgt tag                                            740
Arg Gly Glu Cys
235
```

<210> 2
<211> 238
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Synthetic sequence

<400> 2

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
  1               5                  10                  15

Val His Ser Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val
            20                  25                  30
```

```
Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Asn Leu
          35                  40                  45

Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro
      50                  55                  60

Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala Ser
 65                  70                  75                  80

Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe Thr
                 85                  90                  95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
            100                 105                 110

Ala Gln Asn Leu Glu Ile Pro Arg Thr Phe Gly Gln Gly Thr Lys Val
        115                 120                 125

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
    130                 135                 140

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145                 150                 155                 160

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
                165                 170                 175

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
            180                 185                 190

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
        195                 200                 205

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
    210                 215                 220

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230                 235
```

<210> 3
<211> 740
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic sequence

<400> 3
ctaacactct cccctgttga agctctttgt gacgggcgag ctcaggccct gatgggtgac 60
ttcgcaggcg tagactttgt gtttctcgta gtctgctttg ctcagcgtca gggtgctgct 120
gaggctgtag gtgctgtcct tgctgtcctg ctctgtgaca ctctcctggg agttacccga 180
ttggagggcg ttatccacct tccactgtac tttggcctct ctgggataga agttattcag 240
caggcacaca acagaggcag ttccagattt caactgctca tcagatggcg ggaagatgaa 300
gacagatggt gcagccaccg tacgtttgat ctccaccttg gtcccttggc cgaacgtccg 360
aggaatctct agattttgag cacagtaata aaccccaaca tcctcagcct ccactctgct 420
gattttcagt gtaaaatctg tgcctgatcc actgctactg aacctgtcag ggacccctga 480
ggcaaggttg gacatctgat agatcaggag ctgtggagac tgccctggct tctgcaggta 540
ccaatacaaa taagtgatgc cattactatg caggagattc ttactagacc tacaggagat 600
ggaggccggc tctccagggg tgacgggcag ggagagtgga gactgagtca tcacaatatc 660
ggagtggaca cctgtagctg ttgctaccaa gaagaggatg atacagctcc atcccatggt 720
gaggtcctgt gaagcttacg 740

<210> 4
<211> 1418
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (24)..(1418)

<220>

<223> Description of Artificial Sequence: Synthetic sequence

<400> 4

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cgtaagcttc acagatcctc acc atg gga tgg agc tgt atc atc ctc ttt ctg 53
                          Met Gly Trp Ser Cys Ile Ile Leu Phe Leu
                            1               5                  10

gtg gca aca gct aca ggt gtc cac tcc cag gta cag cta gtg caa tca   101
Val Ala Thr Ala Thr Gly Val His Ser Gln Val Gln Leu Val Gln Ser
                  15                  20                  25

ggg cct gaa gtg aag aag cct ggg gcc tca gtg aaa gtt tcc tgc aag   149
Gly Pro Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys
              30                  35                  40

gct tct ggc tac acc ttc acc aac tat gga atg aac tgg gta agg cag   197
Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Arg Gln
          45                  50                  55

gcg cct gga cag ggg ctt gag tgg atg ggg tgg ata aac acc tac act   245
Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr
      60                  65                  70

gga gag cca aca tat ggt gaa gat ttc aag gga cgg ttt gca ttc tct   293
Gly Glu Pro Thr Tyr Gly Glu Asp Phe Lys Gly Arg Phe Ala Phe Ser
  75                  80                  85                  90

cta gac aca tcc gcc agc aca gcc tat atg gag ctc agc tcg ctg aga   341
Leu Asp Thr Ser Ala Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg
                  95                 100                 105

tcc gag gac act gca gtc tat ttc tgt gcg aga ttt ggt aac tac gta   389
Ser Glu Asp Thr Ala Val Tyr Phe Cys Ala Arg Phe Gly Asn Tyr Val
              110                 115                 120
```

```
gac tac tgg ggt caa gga tca cta gtc act gtc tcc tca gcc tcc acc   437
Asp Tyr Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser Ala Ser Thr
        125                 130                 135

aag ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc aag agc acc tct   485
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser
    140                 145                 150

ggg ggc aca gcg gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa   533
Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
155                 160                 165                 170

ccg gtg acg gtg tcg tgg aac tca ggc gcc ctg acc agc ggc gtg cac   581
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
                175                 180                 185

acc ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc ctc agc agc   629
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
            190                 195                 200

gtg gtg acc gtg ccc tcc agc agc ttg ggc acc cag acc tac atc tgc   677
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys
        205                 210                 215

aac gtg aat cac aag ccc agc aac acc aag gtg gac aag aaa gtt gag   725
Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu
    220                 225                 230

ccc aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca gca cct   773
Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
235                 240                 245                 250

gaa ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag   821
Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
                255                 260                 265
```

```
gac acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg   869
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
            270                 275                 280

gac gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac   917
Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
        285                 290                 295

ggc gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac   965
Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
    300                 305                 310

aac agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac   1013
Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
315                 320                 325                 330

tgg ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc   1061
Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
                335                 340                 345

cca gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga   1109
Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
            350                 355                 360

gaa cca cag gtg tac acc ctg ccc cca tcc cgg gat gag ctg acc aag   1157
Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys
        365                 370                 375

aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tat ccc agc gac   1205
Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
    380                 385                 390

atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac aac tac aag   1253
Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
395                 400                 405                 410
```

```
acc acg cct ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc   1301
Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
                415                 420                 425

aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac gtc ttc tca   1349
Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser
            430                 435                 440

tgc tcc gtg atg cat gag gct ctg cac aac cac tac acg cag aag agc   1397
Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
        445                 450                 455

ctc tcc ctg tct ccg ggt aaa                                      1418
Leu Ser Leu Ser Pro Gly Lys
    460                 465
```

<210> 5
<211> 465
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Synthetic sequence

<400> 5

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
  1               5                  10                  15

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
            20                  25                  30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
        35                  40                  45

Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
    50                  55                  60
```

Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Gly
65 70 75 80

Glu Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Ala Ser
85 90 95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Phe Cys Ala Arg Phe Gly Asn Tyr Val Asp Tyr Trp Gly Gln Gly
115 120 125

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
130 135 140

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
145 150 155 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
165 170 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
180 185 190

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
195 200 205

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
210 215 220

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
225 230 235 240

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
245 250 255

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
260 265 270

```
Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
        275                 280                 285

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
    290                 295                 300

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
305                 310                 315                 320

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
                325                 330                 335

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
            340                 345                 350

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
        355                 360                 365

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
    370                 375                 380

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
385                 390                 395                 400

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
                405                 410                 415

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
            420                 425                 430

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
        435                 440                 445

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
    450                 455                 460

Lys
465
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<210> 6
<211> 1418
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (24)..(1412)

<220>
<223> Description of Artificial Sequence: Synthetic sequence

<400> 6

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                          Met Gly Trp Ser Cys Ile Ile Leu Phe Leu
                            1               5                  10

gtg gca aca gct aca ggt gtc cac tcc cag gta cag cta gtg caa tca   101
Val Ala Thr Ala Thr Gly Val His Ser Gln Val Gln Leu Val Gln Ser
                 15                  20                  25

ggg cct gaa gtg aag aag cct ggg gcc tca gtg aaa gtt tcc tgc aag   149
Gly Pro Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys
             30                  35                  40

gct tct ggc tac acc ttc acc aac tat gga atg aac tgg gta agg cag   197
Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Met Asn Trp Val Arg Gln
         45                  50                  55

gcg cct gga cag ggg ctt gag tgg atg ggg tgg ata aac acc tac act   245
Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr
     60                  65                  70

gga gag cca aca tat ggt gaa gat ttc aag gga cgg ttt gca ttc tct   293
Gly Glu Pro Thr Tyr Gly Glu Asp Phe Lys Gly Arg Phe Ala Phe Ser
 75                  80                  85                  90
```

```
cta gac aca tcc gcc agc aca gcc tat atg gag ctc agc tcg ctg aga   341
Leu Asp Thr Ser Ala Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg
                95                  100                 105

tcc gag gac act gca gtc tat ttc tgt gcg aga ttt ggt aac tac gta   389
Ser Glu Asp Thr Ala Val Tyr Phe Cys Ala Arg Phe Gly Asn Tyr Val
            110                 115                 120

gac tac tgg ggt caa gga tca cta gtc act gtc tcc tca gct tcc acc   437
Asp Tyr Trp Gly Gln Gly Ser Leu Val Thr Val Ser Ser Ala Ser Thr
        125                 130                 135

aag ggc cca tcc gtc ttc ccc ctg gcg ccc tgc tcc agg agc acc tcc   485
Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser
    140                 145                 150

gag agc aca gcc gcc ctg ggc tgc ctg gtc aag gac tac ttc ccc gaa   533
Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
155                 160                 165                 170

ccg gtg acg gtg tcg tgg aac tca ggc gcc ctg acc agc ggc gtg cac   581
Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His
                175                 180                 185

acc ttc ccg gct gtc cta cag tcc tca gga ctc tac tcc ctc agc agc   629
Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
            190                 195                 200

gtg gtg acc gtg ccc tcc agc agc ttg ggc acg aag acc tac acc tgc   677
Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys
        205                 210                 215

aac gta gat cac aag ccc agc aac acc aag gtg gac aag aga gtt gag   725
Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu
    220                 225                 230
```

```
tcc aaa tat ggt ccc cca tgc cca ccg tgc cct gca cct gag ttc gcg   773
Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro Glu Phe Ala
235                 240                 245                 250

ggg gca cca tca gtc ttc ctg ttc ccc cca aaa ccc aag gac act ctc   821
Gly Ala Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
                255                 260                 265

atg atc tcc cgg acc cct gag gtc acg tgc gtg gtg gtg gac gtg agc   869
Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
            270                 275                 280

cag gaa gac ccc gag gtc cag ttc aac tgg tac gtg gat ggc gtg gag   917
Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu
        285                 290                 295

gtg cat aat gcc aag aca aag ccg cgg gag gag cag ttc aac agc acg   965
Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr
    300                 305                 310

tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg ctg acc   1013
Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Thr
315                 320                 325                 330

ggc aag gcg tac aag tgc aag gtc tcc aac aaa ggc ctc ccg tcc tcc   1061
Gly Lys Ala Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser
                335                 340                 345

atc gag aaa acc atc tcc aaa gcc aaa ggg cag ccc cga gag cca cag   1109
Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
            350                 355                 360

gtg tac acc ctg ccc cca tcc cag gag gag atg acc aag aac cag gtc   1157
Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys Asn Gln Val
        365                 370                 375
```

```
agc ctg acc tgc ctg gtc aaa ggc ttc tac ccc agc gac atc gcc gtg   1205
Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
    380                 385                 390

gag tgg gag agc aat ggg cag ccg gag aac aac tac aag acc acg cct   1253
Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro
395                 400                 405                 410

ccc gtg ctg gac tcc gac ggc tcc ttc ttc ctc tac agc agg cta acc   1301
Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr
                415                 420                 425

gtg gac aag agc agg tgg cag gag ggg aat gtc ttc tca tgc tcc gtg   1349
Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser Cys Ser Val
            430                 435                 440

atg cat gag gct ctg cac aac cac tac aca cag aag agc ctc tgc ctg   1397
Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Cys Leu
        445                 450                 455

tct ctg ggt aaa tga gaattc                                       1418
Ser Leu Gly Lys
    460
```

<210> 7
<211> 462
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Synthetic sequence

<400> 7

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
  1               5                  10                  15

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
            20                  25                  30
```

```
Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
          35                  40                  45

Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
      50                  55                  60

Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Gly
 65                  70                  75                  80

Glu Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Ala Ser
                 85                  90                  95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
            100                 105                 110

Tyr Phe Cys Ala Arg Phe Gly Asn Tyr Val Asp Tyr Trp Gly Gln Gly
        115                 120                 125

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
    130                 135                 140

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
145                 150                 155                 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
                165                 170                 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
            180                 185                 190

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
        195                 200                 205

Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro
    210                 215                 220

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro
225                 230                 235                 240
```

Cys Pro Pro Cys Pro Ala Pro Glu Phe Ala Gly Ala Pro Ser Val Phe
245 250 255

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
260 265 270

Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val
275 280 285

Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
290 295 300

Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val
305 310 315 320

Leu Thr Val Leu His Gln Asp Trp Leu Thr Gly Lys Ala Tyr Lys Cys
325 330 335

Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser
340 345 350

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
355 360 365

Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
370 375 380

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
385 390 395 400

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
405 410 415

Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp
420 425 430

Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
435 440 445

Asn His Tyr Thr Gln Lys Ser Leu Cys Leu Ser Leu Gly Lys
450 455 460

<210> 8
<211> 1392
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (58)..(1386)

<220>
<223> Description of Artificial Sequence: Synthetic sequence

<400> 8
atggattggc tgtggaactt gctattcctg atggcagctg cccaaagtat ccaagca 57

cag atc cag ttg gtg cag tct gga cct gaa ctg aag aag cct gga gag 105
Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
1 5 10 15

aca gtc aag atc tcc tgc aag gct tct gga tat acc ttc aca aac tat 153
Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

gga atg aac tgg gtg agg cag gct tca gga gag ggt tta aag tgg atg 201
Gly Met Asn Trp Val Arg Gln Ala Ser Gly Glu Gly Leu Lys Trp Met
35 40 45

ggc tgg ata aac acc tac act gga gag cca aca tat ggt gaa gat ttc 249
Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Gly Glu Asp Phe
50 55 60

```
aag gga cgg ttt gcc ttc tct ttg gaa acc tct gcc agc act gcc tat   297
Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
 65                  70                  75                  80

ttg cag atc aac aac ctc aaa aat gaa gac acg gct aca tat ttc tgt   345
Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
                85                  90                  95

gca aga ttt ggt aac tac gta gac tac tgg ggc caa ggc acc act ctc   393
Ala Arg Phe Gly Asn Tyr Val Asp Tyr Trp Gly Gln Gly Thr Thr Leu
            100                 105                 110

aca gtc tcc tca gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg gcg   441
Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
        115                 120                 125

ccc tgc tcc agg agc acc tcc gag agc aca gcg gcc ctg ggc tgc ctg   489
Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu
    130                 135                 140

gtc aag gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc   537
Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145                 150                 155                 160

gct ctg acc agc ggc gtg cac acc ttc cca gct gtc cta cag tcc tca   585
Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
                165                 170                 175

gga ctc tac tcc ctc agc agc gtg gtg acc gtg ccc tcc agc aac ttc   633
Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe
            180                 185                 190

ggc acc cag acc tac acc tgc aac gta gat cac aag ccc agc aac acc   681
Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr
        195                 200                 205
```

```
aag gtg gac aag aca gtt gag cgc aaa tgt tgt gtc gag tgc cca ccg   729
Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro
    210                 215                 220

tgc cca gca cca cct gtg gca gga ccg tca gtc ttc ctc ttc ccc cca   777
Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro
225                 230                 235                 240

aaa ccc aag gac acc ctc atg atc tcc cgg acc cct gag gtc acg tgc   825
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
                245                 250                 255

gtg gtg gtg gac gtg agc cac gaa gac ccc gag gtc cag ttc aac tgg   873
Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp
            260                 265                 270

tac gtg gac ggc gtg gag gtg cat aat gcc aag aca aag cca cgg gag   921
Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
        275                 280                 285

gag cag ttc aac agc acg ttc cgt gtg gtc agc gtc ctc acc gtt gtg   969
Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val
    290                 295                 300

cac cag gac tgg ctg aac ggc aag gag tac aag tgc aag gtc tcc aac   1017
His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
305                 310                 315                 320

aaa ggc ctc cca gcc ccc atc gag aaa acc atc tcc aaa acc aaa ggg   1065
Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly
                325                 330                 335

cag ccc cga gaa cca cag gtg tac acc ctg ccc cca tcc cgg gag gag   1113
Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
            340                 345                 350
```

```
atg acc aag aac cag gtc agc ctg acc tgc ctg gtc aaa ggc ttc tac   1161
Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
        355                 360                 365

ccc agc gac atc gcc gtg gag tgg gag agc aat ggg cag ccg gag aac   1209
Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
    370                 375                 380

aac tac aag acc aca cct ccc atg ctg gac tcc gac ggc tcc ttc ttc   1257
Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe
385                 390                 395                 400

ctc tac agc aag ctc acc gtg gac aag agc agg tgg cag cag ggg aac   1305
Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
                405                 410                 415

gtc ttc tca tgc tcc gtg atg cat gag gct ctg cac aac cac tac aca   1353
Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
            420                 425                 430

cag aag agc ctc tgc ctg tct ctg ggt aaa tga gaattc               1392
Gln Lys Ser Leu Cys Leu Ser Leu Gly Lys
        435                 440
```

<210> 9
<211> 442
<212> PRT
<213> Artificial Sequence
<223> Description of Artificial Sequence: Synthetic sequence

<400> 9

```
Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
  1               5                  10                  15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
            20                  25                  30
```

```
Gly Met Asn Trp Val Arg Gln Ala Ser Gly Glu Gly Leu Lys Trp Met
          35                  40                  45

Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Gly Glu Asp Phe
      50                  55                  60

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
 65                  70                  75                  80

Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
                 85                  90                  95

Ala Arg Phe Gly Asn Tyr Val Asp Tyr Trp Gly Gln Gly Thr Thr Leu
             100                 105                 110

Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
         115                 120                 125

Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu
     130                 135                 140

Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145                 150                 155                 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
                165                 170                 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Asn Phe
             180                 185                 190

Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr
         195                 200                 205

Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro
     210                 215                 220

Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro
225                 230                 235                 240
```

```
Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
                245                 250                 255

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp
            260                 265                 270

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
        275                 280                 285

Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val
    290                 295                 300

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
305                 310                 315                 320

Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly
                325                 330                 335

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
            340                 345                 350

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
        355                 360                 365

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
    370                 375                 380

Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe
385                 390                 395                 400

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
                405                 410                 415

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
            420                 425                 430

Gln Lys Ser Leu Cys Leu Ser Leu Gly Lys
        435                 440
```

<210> 10
<211> 1392
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic sequence

<400> 10
gaattctcat ttacccagag acaggcagag gctcttctgt gtgtagtggt tgtgcagagc 60
ctcatgcatc acggagcatg agaagacgtt cccctgctgc cacctgctct tgtccacggt 120
gagcttgctg tagaggaaga aggagccgtc ggagtccagc atgggaggtg tggtcttgta 180
gttgttctcc ggctgcccat tgctctccca ctccacggcg atgtcgctgg ggtagaagcc 240
tttgaccagg caggtcaggc tgacctggtt cttggtcatc tcctcccggg atgggggcag 300
ggtgtacacc tgtggttctc ggggctgccc tttggttttg gagatggttt tctcgatggg 360
ggctgggagg cctttgttgg agaccttgca cttgtactcc ttgccgttca gccagtcctg 420
gtgcacaacg gtgaggacgc tgaccacacg gaacgtgctg ttgaactgct cctcccgtgg 480
ctttgtcttg gcattatgca cctccacgcc gtccacgtac cagttgaact ggacctcggg 540
gtcttcgtgg ctcacgtcca ccaccacgca cgtgacctca ggggtccggg agatcatgag 600
ggtgtccttg ggttttgggg ggaagaggaa gactgacggt cctgccacag gtggtgctgg 660
gcacggtggg cactcgacac aacatttgcg ctcaactgtc ttgtccacct tggtgttgct 720
gggcttgtga tctacgttgc aggtgtaggt ctgggtgccg aagttgctgg agggcacggt 780
caccacgctg ctgagggagt agagtcctga ggactgtagg acagctggga aggtgtgcac 840
gccgctggtc agagcgcctg agttccacga caccgtcacc ggttcgggga agtagtcctt 900
gaccaggcag cccagggccg ctgtgctctc ggaggtgctc ctggagcagg gcgccagggg 960
gaagaccgat gggcccttgg tggaggctga ggagactgtg agagtggtgc cttggcccca 1020
gtagtctacg tagttaccaa atcttgcaca gaaatatgta gccgtgtctt catttttgag 1080
gttgttgatc tgcaaatagg cagtgctggc agaggtttcc aaagagaagg caaaccgtcc 1140
cttgaaatct tcaccatatg ttggctctcc agtgtaggtg tttatccagc ccatccactt 1200
taaaccctct cctgaagcct gcctcaccca gttcattcca tagtttgtga aggtatatcc 1260
agaagccttg caggagatct tgactgtctc tccaggcttc ttcagttcag gtccagactg 1320
caccaactgg atctgtgctt ggatactttg ggcagctgcc atcaggaata gcaagttcca 1380
cagccaatcc at 1392

<210> 11
<211> 238
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic sequence

<400> 11

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
 1               5                  10                  15

Val His Ser Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val
            20                  25                  30

Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Asn Leu
        35                  40                  45

Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro
    50                  55                  60

Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala Ser
65                  70                  75                  80

Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe Thr
                85                  90                  95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
            100                 105                 110

Ala Gln Asn Leu Glu Ile Pro Arg Thr Phe Gly Gln Gly Thr Lys Val
        115                 120                 125

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
    130                 135                 140
```

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145 150 155 160

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
165 170 175

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
180 185 190

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
195 200 205

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
210 215 220

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 12
<211> 465
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic sequence

<400> 12
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

```
Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
     50                  55                  60

Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Gly
 65                  70                  75                  80

Glu Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Ala Ser
                 85                  90                  95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
            100                 105                 110

Tyr Phe Cys Ala Arg Phe Gly Asn Tyr Val Asp Tyr Trp Gly Gln Gly
        115                 120                 125

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
    130                 135                 140

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
145                 150                 155                 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
                165                 170                 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
            180                 185                 190

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
        195                 200                 205

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
    210                 215                 220

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
225                 230                 235                 240

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
                245                 250                 255
```

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
260 265 270

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
275 280 285

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
290 295 300

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
305 310 315 320

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
325 330 335

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
340 345 350

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
355 360 365

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
370 375 380

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
385 390 395 400

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
405 410 415

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
420 425 430

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
435 440 445

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
450 455 460

Lys
465

<210> 13
<211> 238
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic sequence

<400> 13

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
  1               5                  10                  15

Val His Ser Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val
             20                  25                  30

Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Asn Leu
         35                  40                  45

Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro
     50                  55                  60

Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala Ser
 65                  70                  75                  80

Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe Thr
                 85                  90                  95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
            100                 105                 110

Ala Gln Asn Leu Glu Ile Pro Arg Thr Phe Gly Gln Gly Thr Lys Val
        115                 120                 125
```

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
130 135 140

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145 150 155 160

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
165 170 175

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
180 185 190

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
195 200 205

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
210 215 220

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 14
<211> 462
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic sequence

<400> 14
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
20 25 30

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Gly
65 70 75 80

Glu Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Ala Ser
85 90 95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Phe Cys Ala Arg Phe Gly Asn Tyr Val Asp Tyr Trp Gly Gln Gly
115 120 125

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
130 135 140

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
145 150 155 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
165 170 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
180 185 190

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
195 200 205

Ser Ser Leu Gly Thr Lys Thr Tyr Thr Cys Asn Val Asp His Lys Pro
210 215 220

Ser Asn Thr Lys Val Asp Lys Arg Val Glu Ser Lys Tyr Gly Pro Pro
225 230 235 240

```
Cys Pro Pro Cys Pro Ala Pro Glu Phe Ala Gly Ala Pro Ser Val Phe
                245                 250                 255

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
            260                 265                 270

Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp Pro Glu Val
        275                 280                 285

Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
    290                 295                 300

Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Tyr Arg Val Val Ser Val
305                 310                 315                 320

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Ala Tyr Lys Cys
                325                 330                 335

Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys Thr Ile Ser
            340                 345                 350

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
        355                 360                 365

Ser Gln Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
    370                 375                 380

Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
385                 390                 395                 400

Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
                405                 410                 415

Gly Ser Phe Phe Leu Tyr Ser Arg Leu Thr Val Asp Lys Ser Arg Trp
            420                 425                 430

Gln Glu Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
        435                 440                 445
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Asn His Tyr Thr Gln Lys Ser Leu Cys Leu Ser Leu Gly Lys
450 455 460

<210> 15
<211> 238
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic sequence

<400> 15
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15

Val His Ser Asp Ile Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val
20 25 30

Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Lys Asn Leu
35 40 45

Leu His Ser Asn Gly Ile Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro
50 55 60

Gly Gln Ser Pro Gln Leu Leu Ile Tyr Gln Met Ser Asn Leu Ala Ser
65 70 75 80

Gly Val Pro Asp Arg Phe Ser Ser Ser Gly Ser Gly Thr Asp Phe Thr
85 90 95

Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys
100 105 110

Ala Gln Asn Leu Glu Ile Pro Arg Thr Phe Gly Gln Gly Thr Lys Val
115 120 125

```
Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro
    130                 135                 140

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu
145                 150                 155                 160

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn
                165                 170                 175

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser
            180                 185                 190

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala
        195                 200                 205

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly
    210                 215                 220

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225                 230                 235
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<210> 16
<211> 461
<212> PRT
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: Synthetic sequence

<400> 16

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  1               5                  10                  15

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Pro Glu Val Lys Lys
            20                  25                  30
```

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Asn Tyr Gly Met Asn Trp Val Arg Gln Ala Pro Gly Gln Gly Leu
50 55 60

Glu Trp Met Gly Trp Ile Asn Thr Tyr Thr Gly Glu Pro Thr Tyr Gly
65 70 75 80

Glu Asp Phe Lys Gly Arg Phe Ala Phe Ser Leu Asp Thr Ser Ala Ser
85 90 95

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val
100 105 110

Tyr Phe Cys Ala Arg Phe Gly Asn Tyr Val Asp Tyr Trp Gly Gln Gly
115 120 125

Ser Leu Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
130 135 140

Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser Thr Ala Ala Leu
145 150 155 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
165 170 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
180 185 190

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
195 200 205

Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val Asp His Lys Pro
210 215 220

Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys Cys Cys Val Glu
225 230 235 240

Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro Ser Val Phe Leu
245 250 255

Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu
260 265 270

Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Gln
275 280 285

Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys
290 295 300

Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val Val Ser Val Leu
305 310 315 320

Thr Val Val His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys
325 330 335

Val Ser Asn Lys Gly Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys
340 345 350

Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser
355 360 365

Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys
370 375 380

Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln
385 390 395 400

Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu Asp Ser Asp Gly
405 410 415

Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln
420 425 430

Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn
435 440 445

His Tyr Thr Gln Lys Ser Leu Cys Leu Ser Leu Gly Lys
450 455 460

Docket No.
PU3513USW

Declaration And Power Of Attorney For Patent Application

English Language Declaration

As below named inventor. I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

COMBINATION OF AN ANTI-EP-CAM ANTIBODY WITH A CHEMOTHERAPEUTIC AGENT

the specification of which (check only one item below):

[ ]is attached hereto.
OR
[X] was filed on _____as United States application Serial No. _____ or **PCT** International

Application Number PCT/EP99/05271 filed July 23, 1999 and was amended on (MM/DD/YYYY) _____(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment specifically referred to above.

I acknowledge the duty to disclose to the United States Patent and Trademark Office all information which is material to patentability as defined in 37 CFR §1.56.

I hereby claim foreign priority benefits under 35, U.S.C. §119 (a)-(d) or §365(b) of any foreign applications(s) for patent or inventor's certificate or 365(a) of any PCT international application which designated at least one country other than the United States of America, listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate or of any PCT international application having a filing date before that of the application on which priority is claimed:

Prior Foreign Application Number (s)	Country	Foreign Filing Date (MM/DD/YYYY))	PRIORITY CLAIMED
1.			
2.			
3.			
4.			
5.			

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date (MM/DD/YYYY)	
1.		
2.		
3.		
4.		
5.		

Express Mail Label No.
EL395894335US

COMBINED DECLARATION FOR UTILITY or DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY Continued

ATTORNEY'S DOCKET NUMBER: PU3513USW

I hereby claim the benefit under 35, U S C §120 of any United States application or §365(c) of any PCT international application designating the United States of America that is listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F R. §1 56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application:

PRIOR U.S. PARENT APPLICATION or PCT PARENT APPLICATION

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	STATUS (Check one) PATENTED	PENDING	ABANDONED
PCT/EP99/05271	07/23/1999		X	

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith. (List name and registration number)

23347
PATENT TRADEMARK OFFICE

Send Correspondence to:

23347
PATENT TRADEMARK OFFICE

Direct Telephone Calls to:

Frank P. Grassler
919-483-2482

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S.C. 1001, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

1-002	FULL NAME OF INVENTOR	FAMILY NAME: KNICK	FIRST GIVEN NAME: Vincent	SECOND GIVEN NAME/INITIAL: C.
	INVENTOR'S SIGNATURE	Vincent C Knick		DATE: 14 Jan 2002
0	RESIDENCE & CITIZENSHIP	CITY: Durham	STATE OR FOREIGN COUNTRY: North Carolina NC	COUNTRY OF CITIZENSHIP: US
1	POST OFFICE ADDRESS	POST OFFICE ADDRESS: GlaxoSmithKline Five Moore Drive, PO Box 13398	CITY: Research Triangle Park	STATE & ZIP CODE/COUNTRY: NC 27709 US
2	FULL NAME OF INVENTOR	FAMILY NAME: STIMMEL	FIRST GIVEN NAME: Julie	SECOND GIVEN NAME/INITIAL: Beth
	INVENTOR'S SIGNATURE			DATE:
0	RESIDENCE & CITIZENSHIP	CITY: Durham	STATE OR FOREIGN COUNTRY: North Carolina	COUNTRY OF CITIZENSHIP: US
2	POST OFFICE ADDRESS	POST OFFICE ADDRESS: GlaxoSmithKline, Inc. Five Moore Drive, PO Box 13398	CITY: Research Triangle Park	STATE & ZIP CODE/COUNTRY: NC 27709 US
2	FULL NAME OF INVENTOR	FAMILY NAME: THURMOND	FIRST GIVEN NAME: Linda	SECOND GIVEN NAME/INITIAL: M.
	INVENTOR'S SIGNATURE			DATE:
0	RESIDENCE & CITIZENSHIP	CITY: Durham	STATE OR FOREIGN COUNTRY: North Carolina	COUNTRY OF CITIZENSHIP: US

[redacted]

Docket No.
PU3513USW

Declaration And Power Of Attorney For Patent Application

English Language Declaration

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My residence, post office address and citizenship are as stated below next to my name.

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COMBINATION OF AN ANTI-EP-CAM ANTIBODY WITH A CHEMOTHERAPEUTIC AGENT

the specification of which (check only one item below):

[ ]is attached hereto.
OR
[X] was filed on _____as United States application Serial No. _____ or **PCT** International

Application Number PCT/EP99/05271 filed July 23, 1999 and was amended on (MM/DD/YYYY) _____(if applicable)

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1.			
2.			
3.			
4.			
5.			

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date (MM/DD/YYYY)	
1.		
2.		
3.		
4.		
5.		

COMBINED DECLARATION FOR UTILITY or DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY Continued

ATTORNEY'S DOCKET NUMBER: PU3513USW

I hereby claim the benefit under 35, U.S.C. §120 of any United States application or §365(c) of any PCT international application designating the United States of America that is listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of 35 U S C §112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C F R §1 56 which became available between the filing date of the prior application(s) and the national or PCT international filing date of this application

PRIOR U.S. PARENT APPLICATION or PCT PARENT APPLICATION

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	STATUS (Check one) PATENTED	PENDING	ABANDONED
PCT/EP99/05271	07/23/1999		X	

POWER OF ATTORNEY: As a named inventor, I hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the U.S. Patent and Trademark Office connected therewith (List name and registration number)

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2	FULL NAME OF INVENTOR	FAMILY NAME: **KNICK**	FIRST GIVEN NAME: **Vincent**	SECOND GIVEN NAME/INITIAL: **C.**
	INVENTOR'S SIGNATURE			**DATE:**
0	RESIDENCE & CITIZENSHIP	CITY: **Durham**	STATE OR FOREIGN COUNTRY: **North Carolina**	COUNTRY OF CITIZENSHIP: **US**
1	POST OFFICE ADDRESS	POST OFFICE ADDRESS: **GlaxoSmithKline Five Moore Drive, PO Box 13398**	CITY: **Research Triangle Park**	STATE & ZIP CODE/COUNTRY: **NC 27709 US**
2	FULL NAME OF INVENTOR	FAMILY NAME: **STIMMEL**	FIRST GIVEN NAME: **Julie**	SECOND GIVEN NAME/INITIAL: **Beth**
20	**INVENTOR'S SIGNATURE**	Julie Beth Stimmel		**DATE:** 14 Jan 02
0	RESIDENCE & CITIZENSHIP	CITY: **Durham**	STATE OR FOREIGN COUNTRY: **North Carolina** NC	COUNTRY OF CITIZENSHIP: **US**
2	POST OFFICE ADDRESS	POST OFFICE ADDRESS: **GlaxoSmithKline, Inc. Five Moore Drive, PO Box 13398**	CITY: **Research Triangle Park**	STATE & ZIP CODE/COUNTRY: **NC 27709 US**
2	FULL NAME OF INVENTOR	FAMILY NAME: **THURMOND**	FIRST GIVEN NAME: **Linda**	SECOND GIVEN NAME/INITIAL: **M.**
	INVENTOR'S SIGNATURE			**DATE:**
0	RESIDENCE & CITIZENSHIP	CITY: **Durham**	STATE OR FOREIGN COUNTRY: **North Carolina**	COUNTRY OF CITIZENSHIP: **US**
3	POST OFFICE ADDRESS	POST OFFICE ADDRESS: **GlaxoSmithKline Five Moore Drive, PO Box 13398**	CITY: **Research Triangle Park**	STATE & ZIP CODE/COUNTRY: **NC 27709 US**

Docket No.
PU3513USW

Declaration And Power Of Attorney For Patent Application

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COMBINATION OF AN ANTI-EP-CAM ANTIBODY WITH A CHEMOTHERAPEUTIC AGENT

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[ ]is attached hereto.
OR
[X] was filed on _____as United States application Serial No. _____ or **PCT** International

Application Number PCT/EP99/05271 filed July 23, 1999 and was amended on (MM/DD/YYYY)
_____(if applicable)

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1.			
2.			
3.			
4.			
5.			

I hereby claim the benefit under Title 35, United States Code §119(e) of any United States provisional application(s) listed below:

Application No.	Filing Date (MM/DD/YYYY)	
1.		
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4.		
5.		

COMBINED DECLARATION FOR UTILITY or DESIGN PATENT APPLICATION WITH POWER OF ATTORNEY Continued

ATTORNEY'S DOCKET NUMBER: PU3513USW

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PRIOR U.S. PARENT APPLICATION or PCT PARENT APPLICATION

U.S. Parent Application or PCT Parent Number	Parent Filing Date (MM/DD/YYYY)	STATUS (Check one) PATENTED	PENDING	ABANDONED
PCT/EP99/05271	07/23/1999		X	

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2	FULL NAME OF INVENTOR	FAMILY NAME: **KNICK**	FIRST GIVEN NAME: **Vincent**	SECOND GIVEN NAME/INITIAL: **C.**
	INVENTOR'S SIGNATURE			**DATE:**
0	RESIDENCE & CITIZENSHIP	CITY: **Durham**	STATE OR FOREIGN COUNTRY: **North Carolina**	COUNTRY OF CITIZENSHIP: **US**
1	POST OFFICE ADDRESS	POST OFFICE ADDRESS: **GlaxoSmithKline Five Moore Drive, PO Box 13398**	CITY: **Research Triangle Park**	STATE & ZIP CODE/COUNTRY: **NC 27709 US**
2	FULL NAME OF INVENTOR	FAMILY NAME: **STIMMEL**	FIRST GIVEN NAME: **Julie**	SECOND GIVEN NAME/INITIAL: **Beth**
	INVENTOR'S SIGNATURE			**DATE:**
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300 2	FULL NAME OF INVENTOR	FAMILY NAME: **THURMOND**	FIRST GIVEN NAME: **Linda**	SECOND GIVEN NAME/INITIAL: **M.**
	INVENTOR'S SIGNATURE	Linda M Thurmond		DATE: 14 Jan 02
0	RESIDENCE & CITIZENSHIP	CITY: **Durham**	STATE OR FOREIGN COUNTRY: **North Carolina** NC	COUNTRY OF CITIZENSHIP: **US**
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